UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: May 4, 2017

SUBJECT: **Sedaxane**: Cancer Reassessment of the Cancer Assessment Review Committee

PC Code: 129223
Decision No.: N/A
Petition No.: N/A
Risk Assessment Type: Cancer Assessment
TXR No.: 0057604
MRID No.: N/A

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FROM: Christopher Schlosser, Executive Secretary
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Health Effects Division (7509P)

5/4/17

THROUGH: Gregory Akerman, Chair
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5/4/17

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The Cancer Assessment Review Committee met on February 22, 2017 to re-evaluate the cancer classification of sedaxane in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the final Cancer Assessment Document.

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL/MODE OF ACTION FOR MOUSE & RAT LIVER TUMORS, RAT THYROID TUMORS, AND RAT UTERINE TUMORS

SEDAXANE

PC Code 129223

May 4, 2017

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE

HEALTH EFFECTS DIVISION

OFFICE OF PESTICIDE PROGRAMS

Contents

I. EXECUTIVE SUMMARY

The Cancer Assessment Review Committee (CARC) met on February 22, 2017 to re-evaluate the cancer classification of sedaxane in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). In 2011, the CARC classified sedaxane as "Likely to be Carcinogenic to Humans." This classification was based on the presence of tumors at multiple sites in two species: liver and thyroid tumors in male rats, uterine tumors in female rats, and liver tumors in male mice. A linear low-dose extrapolation model (Q_1*) was used for quantification of cancer risk to humans, with a $Q_1^* = 4.64 \times 10^{-3}$ $(mg/kg/day)^{-1}$ based on male rat thyroid follicular cell tumors, the most potent unit risk. Since that time, Syngenta has submitted 17 new studies to support proposed modes of action for liver, thyroid, and uterine tumors. On February 22, 2017, the CARC convened to evaluate these submissions and to determine a cancer classification based on a weight of evidence of the available data.

The new studies were considered in the context of the registrant's proposed MOA for the liver tumors in male rats and mice, thyroid tumors in male rats, and uterine tumors in female rats. The registrant proposed a constitutive androstane receptor/pregnane-X receptor (CAR/PXR)-mediated mitogenic mode of action for liver tumors in male mice and rats and a liver-mediated altered thyroid hormone homeostasis mode of action for thyroid tumors in male rats. The proposed mode of action for uterine tumors in rats is initiated by a decrease in body weight gain resulting in decreased adipose tissue, leading to a suppression of the age-related decrease in dopaminergic signaling and suppression of prolactin, and subsequent increased age at reproductive senescence.

The CARC concluded that the *in vitro* and *in vivo* data adequately demonstrated dose and temporal concordance to support the key events for the modes of action leading to liver and thyroid tumors in males. However, the CARC concluded that the data do not adequately demonstrate a link between decreased body weight gain and prolactin suppression leading to the increased incidence of uterine tumors in rats.

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), CARC concludes that sedaxane should be reclassified as "Suggestive Evidence of Carcinogenic Potential" based on uterine tumors in female rats (one sex/one species). There is insufficient evidence to support the proposed uterine tumor MOA in female rats.

The rationale for this decision is based on the following considerations:

- The liver and thyroid tumor response induced by sedaxane occurred in only male rats and/or mice; no liver or thyroid tumors were seen in female rats or mice.
- The liver tumor response in male rats occurred late in the course of treatment and was driven by adenomas; no carcinomas were observed. It was considered to be weak evidence of a treatment-related effect.

- The liver tumor response in male mice was driven by adenomas and combined adenomas and/or carcinomas. However, all non-neoplastic histopathology findings were considered background findings associated with the age and strain of mice.

- The thyroid tumor response in male rats was driven mainly by adenomas, although there was also an increase in combined adenomas and/or carcinomas. It was concluded that thyroid tumors were weak evidence of a treatment-related effect.

- There is no concern for mutagenicity. The available mutagenicity studies for sedaxane were negative.

- Data are sufficient to support the proposed MOA for liver tumors in male rats and mice and thyroid tumors in male rats. However, data are not sufficient to support the proposed MOA for female rat uterine tumors.

Based on this classification, the quantification of cancer risk using a Q1* approach is not required. A non-linear approach (i.e., RfD) would adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to sedaxane.

As a follow-up to the February CARC meeting, the CARC met again on March 8, 2017, to clarify the tumorigenic dose level for uterine tumors. The original CARC report (Kidwell, 2012; TXR #0055706) stated that uterine tumors were treatment-related in female rats, but the dose level was not explicitly stated. The conclusions from this second meeting will be addressed in more detail in Section XI.

II. BACKGROUND

The Cancer Assessment Review Committee (CARC) met on February 22, 2017 to re-evaluate the cancer classification of sedaxane in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). This is the second time that sedaxane has been presented to the CARC. The first meeting took place on March 16, 2011, for evaluation of the carcinogenic potential of sedaxane (Kidwell, 2012; TXR #0055706). At that time the CARC classified sedaxane as "Likely to Be Carcinogenic to Humans" based on the following conclusions:

1) Liver adenomas were considered to be treatment-related only at the high dose (3600 ppm) in male rats;
2) Liver tumors were considered to be treatment-related only at the high dose (7000 ppm) in male mice;
3) Thyroid follicular cell tumors were considered to be treatment-related effect only at the high dose (3600 ppm) in male rats; and
4) Uterine tumors were considered to be treatment-related in female rats.

Since that time, Syngenta has submitted 17 new studies addressing these issues and overview documents detailing a human relevance framework analysis for each tumor type (liver, thyroid, and uterine tumors). On February 22, 2017, the CARC reconvened to evaluate these submissions, which included the following studies:

1) CAR3 Transactivation Assay with Mouse, Rat and Human CAR (MRID 49804825);
2) Pregnane X Receptor (PXR) Trans-activation Assays with Rat, Mouse and Human PXR (MRID 49804824);
3) 28 Day Oral (Dietary) Mechanistic Study to Evaluate Effects on the Liver and Thyroid in the Male Rat (MRID 49804819);
4) Method Validation of Radioimmunoassay Analysis of Control Rat Serum for TSH (MRID 49804820);
5) 21 Day Dietary Liver Mode of Action Study in Male CD-1 Mice (MRID 49804810);
6) Enzyme and DNA Synthesis Induction in Cultured Male Human Hepatocytes (MRID 49804811);
7) Enzyme and DNA Synthesis Induction in Cultured Male Han Wistar Rat Hepatocytes (MRID 49804812);
8) 14 Day Range Finding Study by Oral (Dietary) Administration in Male CD-1 Mice (MRID 49804822);
9) Hepatic Enzyme Activities after 28 and 90 Days of Dietary Administration to Male CD-1 Mice (MRID 49804823);
10) *In vivo* Unscheduled DNA Synthesis in Rat Hepatocytes (MRID 50102201);
11) Effect on Rat Thyroid Peroxidase Activity *in Vitro* (MRID 49804821);
12) Uterotrophic Assay in Ovariectomized Wistar Han Rats (MRID 49804803);
13) Microscopic Evaluation of Vagina, Uterus, and Ovary from Subchronic and Chronic Rat Dietary Studies to Determine Cycle Stage (MRID 49804814);

14) Isopyrazam – Evaluation of Hypothalamic Tyrosine Hydroxylase in Control Female Wistar Rats at 3, 12 or 24 Months by Immunohistochemistry and In-situ Hybridization (MRID 49804815);
15) Analysis of Stored Tissue from 2-Year Rat Study for Hypothalamic Tyrosine Hydroxylase *via* Immunohistochemistry and In-Situ Hybridization (MRID 49804816);
16) *In vitro* Dopamine D2S Receptor Binding Assay (MRID 49804817);
17) Sedaxane – Analysis of Prolactin, Leptin and Adiponectin in Serum Samples from a One-Year Sacrifice of Female Wistar Rats (MRID 50101901);
18) Sedaxane – Mode of Action and Human Relevance Assessment of Liver Tumor Incidences in Rats and Mice (MRID 49804809);
19) Sedaxane – Mode of Action and Human Relevance Assessment of Thyroid Follicular Cell Tumors in Male Rats (MRID 49804818); and
20) Sedaxane – Mode of Action and Human Relevance Assessment Uterine Tumors in Female Han Wistar Rats (MRID 49804813).

These new studies were considered when reevaluating the data to support the proposed MOA for the liver tumors in mice and rats and thyroid and uterine tumors in rats.

III. EVALUATION OF LIVER TUMORS AND MECHANISTIC STUDIES

Throughout this document, previously reviewed liver (male rats and mice), thyroid (male rats), and uterine (female rats) tumor data (TXR #0055706) will be briefly discussed separately followed by the proposed mode of action for each tumor type.

The CARC considered the liver adenomas to be weak evidence of a treatment-related effect only at the high dose in male rats (3600 ppm) and mice (7000 ppm), based on the following information:

A1. Liver Tumors in Male <u>Rats</u>:

The following text in Section III. A1.-B3. was extracted directly from the first CARC meeting (March 16, 2011) report (TXR #0055706).

In a combined chronic toxicity/carcinogenicity study, 52 Crl:WI(Han)(Han Wistar) rats/sex/dose were exposed to sedaxane (95.3% a.i.) for up to 2 years in the diet at concentrations of 0, 200, 1200, or 3600 ppm (equivalent to 0/0, 11/14, 67/86, and 218/261 mg/kg bw/day in males/females, respectively) (MRID 47473386). An additional 12 rats/sex/dose were treated similarly for up to 1 year and then sacrificed.

Data from the tumor analyses are presented in **Table 1**. In male Wistar rats, liver tumors were limited to adenomas; no carcinomas were seen. Although a statistically significant trend (Exact Test for Trend) was seen for liver adenomas at $p<0.05$, there were no significant pair-wise comparisons (Fisher's Exact Test) of the dosed groups with the controls. No precursor lesions of the liver were seen in males at this dose. No liver tumors were seen in female rats.

Table 1. Sedaxane – Crl:WI(Han)(Han Wistar) Male Rat Liver Tumor Rates[+]

	Dose (ppm)			
	0	**200**	**1200**	**3600**
Adenomas#	1/52	1[a]/51	1/52	5/52
(%)	(2%)	(2%)	(2%)	(10%)
p =	0.01656*	0.74757	0.75243	0.10248

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.
#No carcinomas were observed.
[a]First adenoma observed at week 89, dose 200 ppm.

Note: Significance of trend denoted at control (p values on the control groups are the trends).
Significance of pair-wise comparison with control (dose 0) denoted at dose level.

A2. Other Related Toxic Effects in Male Rats

In addition to the liver tumors discussed above, a statistically significant dose-related increase in absolute and adjusted liver weight (adjusted for body weight by covariate analysis) was seen at 1200 ppm in both sexes. In males, this effect was associated with increased hepatocyte hypertrophy and eosinophilic foci. At the high-dose level, minimal to moderate centrilobular hepatocyte hypertrophy and increased incidence of pigment in centrilobular or mid-zonal hepatocytes in the liver were seen at week 52 in both sexes and at week 104 only in females. Increased serum gamma glutamyl transferase (GGT), glucose, and phosphate levels were observed in males at 3600 ppm.

A3. CARC Conclusions on Male Rats:

From these data, the CARC considered the liver adenomas to be evidence of a treatment-related effect only at the high dose (3600 ppm) in male rats.

B1. Liver Tumors in Male Mice (extracted from TXR #0055706):

In a carcinogenicity study, sedaxane (95.3% a.i.) was administered in diet to groups of CD-1 mice (50/sex/group) at dose levels of 0, 200, 1250 or 7000 ppm (0, 25, 157, and 900 mg/kg/day for males and 0, 29, 185, and 1001 mg/kg/day for females) for 80 weeks (MRID 47473388).

Data from the tumor analyses are presented in **Table 2**. Male mice had statistically significant dose trends and significant pair-wise comparisons between the 7000 ppm dose group and the controls for hepatocellular adenomas and combined adenomas and/or carcinomas, all at $p < 0.05$. There was also a statistically significant trend only in hepatocellular carcinomas at $p < 0.05$. The

statistical analyses of the tumors presented in **Table 2** in the male mice were based upon Fisher's Exact Test for pair-wise comparisons and the Exact Test for trend (L. Brunsman, TXR #0055689). No liver tumors were seen in female mice.

Table 2. Sedaxane – Crl:CD-1(ICR) Male Mice Liver Tumor Rates[+]

	Dose (ppm)			
	0	**200**	**1250**	**7000**
Adenomas (%)	7/48 (15%)	9[a]/45 (20%)	10/45 (22%)	15/48 (31%)
p =	0.03257*	0.33833	0.24710	0.04389*
Carcinomas (%)	5/48 (10%)	5/45 (11%)	3/45 (7%)	10[b]/48 (21%)
p =	0.03355*	0.58799	0.84447	0.13028
Combined (%)	9[c]/48 (19%)	13[d]/45 (29%)	12[d]/45 (27%)	19[e]/48 (40%)
p =	0.02295*	0.18263	0.25329	0.02113*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 49.

[a]First adenoma observed at week 49, dose 200 ppm.
[b]First carcinoma observed at week 67, dose 7000 ppm.
[c]Three animals in the control group had both an adenoma and a carcinoma.
[d]One animal in each of the 200 and 1250 ppm dose groups had both an adenoma and a carcinoma.
[e]Six animals in the 7000 ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

B2. Other Related Toxic Effects in Male Mice:

No treatment-related neoplastic or non-neoplastic histopathological changes of the liver were observed at 200 or 1250 ppm in male or female rats. Liver weight (covariate analysis) at 7000 ppm was statistically significantly higher in males than the control value (+16%). Dietary administration of sedaxane at 7000 ppm resulted in treatment-related lower body weights and body weight gains for males and females. Dosing was considered to be adequate based on decreased body weight/gains and decreased food utilization in the initial stages of the study.

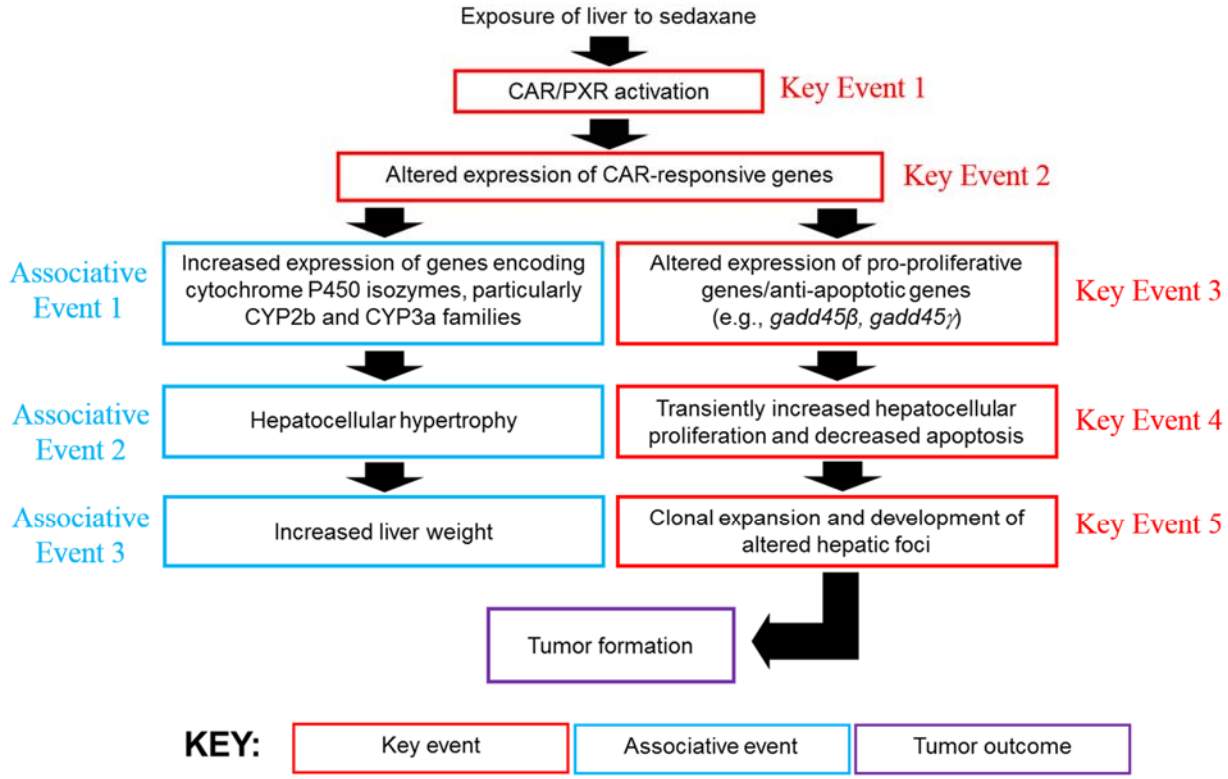
B3. CARC Conclusions on Male Mice:

From these data, the CARC considered the liver tumors to be treatment-related only at the high dose (7000 ppm) in male mice.

C. Proposed Mode of Action for Rodent Liver Tumors

The following mode of action has been postulated by the Registrant (MRID 49804809) for sedaxane-induced mouse and rat liver tumors, which begins with activation of the constitutive androstane receptor (CAR) +/- pregnane-X receptor (PXR). The key events in the proposed MOA include: activation of the constitutive androstane receptor (CAR) +/- pregnane-X receptor (PXR); altered expression of CAR-responsive genes that promote a pro-proliferative and anti-apoptotic environment in the liver; an early, transient increase in hepatocellular proliferation; increased hepatocellular foci as a result of clonal expansion of spontaneously mutated (initiated) cells; and slight increases in liver tumor incidence compared to concurrent controls (**Figure 1**).

Figure 1: Mode of Action Hypothesis for Induction of Liver Tumors in Rats and Mice



1. Mechanistic studies submitted to support proposed MOA

a) CAR and PXR activation assays (Key Event 1)

In an *in vitro* mechanistic study (MRID 49804825), the potential for sedaxane (95.3%) to directly activate CAR (NR1I3) in a reporter assay was studied. Briefly, cDNA expression vectors for CAR3 variants of mouse, rat, and human CAR were transfected into COS-1 cells, along with necessary cofactors and a CYP2B6 response element-luciferase reporter construct. After a suitable expression time, the cells were incubated with sedaxane at concentrations of 1, 3, 10, and 30 µM. The direct CAR activator artemisinin (positive control) was also incubated at these

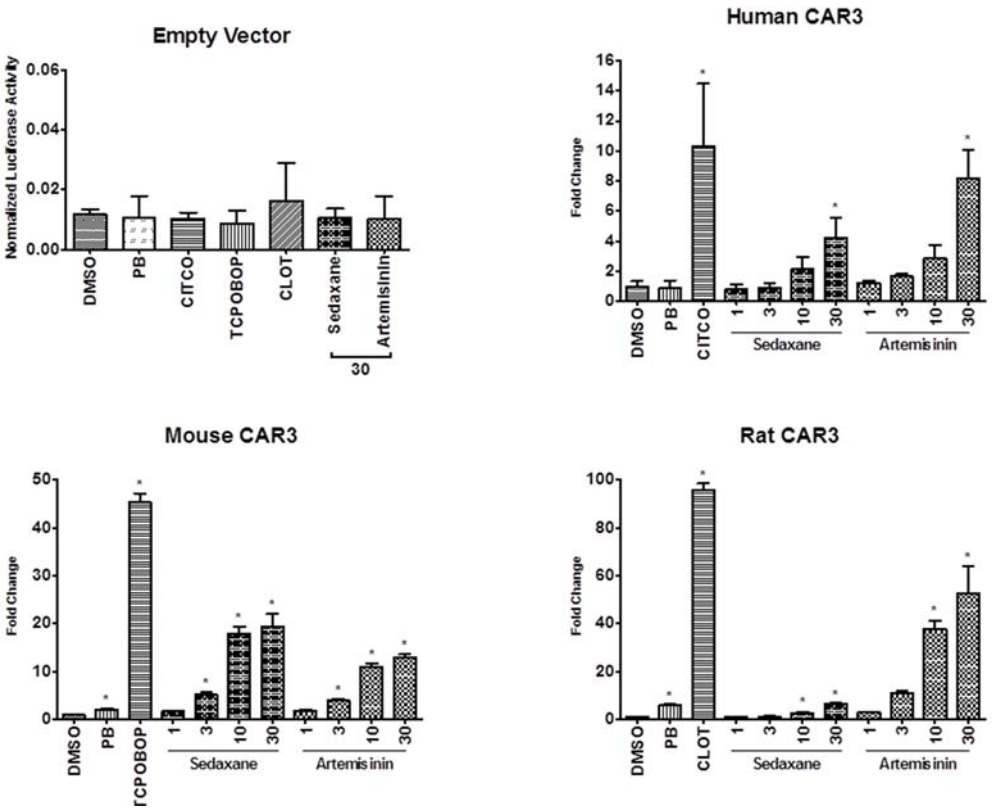
same concentrations, and model direct-acting substrates for mouse, rat, or human CAR were each incubated at a single concentration. Light emission from the luciferase reporter was quantified to indicate the extent of CAR activation upon incubation with suspected ligands, including sedaxane. Results were reported as normalized luciferase activity and fold change compared to a DMSO solvent control.

Sedaxane was tested in the human, mouse, and rat assays, using the respective species' CAR3 reporter constructs. A concentration-dependent activation of mouse CAR3 by sedaxane was observed, with up to 19-fold activation of mouse CAR3. At similar concentrations, sedaxane produced up to a 6-fold activation of rat CAR3. The human CAR3 response was only statistically significant at 30 μM, the highest dose tested, and this response represented a 4-fold activation above solvent control (**Figure 2**).

The model activators CITCO, TCPOBOP, and clotrimazole produced robust responses in human, mouse, and rat CAR3 constructs, respectively. Artemisinin also was tested and produced a concentration-dependent response that was much more marked with rat CAR3 than with human or mouse CAR3 (**Figure 2**).

In summary, these data suggest that sedaxane is a direct activator of mouse, rat, and human CAR. Under the conditions of this study, the activation of mouse CAR was stronger than the activation of rat or human CAR.

Figure 2: Human, Mouse, and Rat CAR3 Reporter Assay Results with Sedaxane and Model Ligands



A second *in vitro* mechanistic study (MRID 49804824) was conducted to evaluate the potential for sedaxane (95.3% wt/wt) to activate the pregnane-X receptor (PXR) of human, rat, and mouse. For the PXR transactivation luciferase assays in human embryonic kidney (HEK) cells, the ligand-binding domain of human, rat, or mouse PXR was fused to the DNA binding domain of the transcription factor Gal4. Concentrations of sedaxane from 14 nM to 30,000 nM were prepared by serial dilution and tested in the PXR reporter assay system. TO901317 (human PXR activator) and pregnenolone-16α-carbonitrile (rat and mouse PXR activator) were also tested at an appropriate range of concentrations as positive control compounds. After 24 hours, the emission of light was measured to quantify the relative light units (RLU), which is a surrogate measure of PXR activity. At each concentration of sedaxane, clotrimazole and the positive/negative controls, cell viability was also assessed using INDIGO's Live Cell Multiplex (LCM) assay.

Sedaxane showed low, but statistically significant, agonist activity in the human PXR assay from 3.33 µM to 30 µM. It also showed a maximum activity approximately 3.9-fold higher than the DMSO vehicle control and was considered to be an activator of human PXR. Sedaxane showed statistically significant activity in the rat PXR assay at concentrations of 41.2 nM to 30 µM. The

maximum activity was approximately 3.1-fold higher than the DMSO vehicle control, and therefore sedaxane was a considered to be an activator of rat PXR. Sedaxane showed no agonist activity in the mouse PXR assay (**Table 3**).

The positive control compounds produced appropriate responses that were species specific. Cell viability was near 100% at all concentrations tested for both sedaxane and the positive/negative controls (data not shown).

TABLE 3: Sedaxane -summary of human, rat and mouse PXR data

		Human PXR Agonist Assays	LCM Assay	Rat PXR Agonist Assays	LCM Assay	Mouse PXR Agonist Assays	LCM Assay
Compound	**nM**	**fold-change**	**% live cells**	**fold-change**	**% live cells**	**fold-change**	**% live cells**
Sedaxane	13.7	0.97	101	1.0	101	1.09	106
	41.2	1.0	101	1.3*	102	1.1	106
	123	1.1	102	1.3*	102	1.0	106
	370	1.0	102	1.3*	101	1.1	106
	1,111	1.3	104	1.3*	104	1.2	106
	3,333	1.7*	105	1.5*	102	1.24	106
	10,000	3.0*	103	1.7*	101	1.2	101
	30,000	3.9*	97	3.1*	99	0.75	97
0.1% DMSO	0.10%	1.0	100	1.0	100	1.0	100

* $p<0.01$

CARC concluded that sedaxane activates mouse, rat and human CAR in addition to rat and human PXR to support Key Event 1 for the proposed mode of action presented in Figure 1.

b) Liver enzyme activity measurements in sedaxane-treated male <u>rats</u> (Associative Event 1)

28 Day Oral (Dietary) Mechanistic Study to Evaluate Effects on the Liver and Thyroid on the Male <u>Rat</u> (MRID 4984819)

Male Han Wistar rats [strain designation Crl:WI(Han); 15 rats/group/time-point] were treated with sedaxane at dietary levels of 0, 1200, and 3600 ppm (equivalent to 0, ~95, ~278 mg/kg bw/day) for 1, 3, 7, 14 or 28 days before termination (Study Days 2, 4, 8, 15 and 29), and a number of liver- and thyroid-related parameters were measured (MRID 49804819). 1200 ppm was the mid-dose and 3600 ppm was the highest dose in the 2-year carcinogenicity study (MRID 47473386). The liver adenomas at the high dose were considered treatment-related in the carcinogenicity study (TXR #0055706). Each parameter measured is discussed in detail below.

The reversibility of sedaxane treatment-related effects on liver- and thyroid-related parameters was assessed by including additional groups of animals treated with either 0 ppm or 3600 ppm sedaxane for 28 days, followed by a 60-day recovery (reversibility) period prior to termination (on Study Day 89). In addition to treatment with sedaxane, further animals were treated with 1200 ppm (equivalent to ~97 mg/kg bw/day) phenobarbital sodium salt (NaPB) as a positive control. The study design was as follows:

Group Number	Treatment	Number of Male Rats					
		Termination Timepoints (Study Day)					
		2	4	8	15	29	89 [R]
1	Control: Basal (untreated) Diet	n=15	n=15	n=15	n=15	n=15	n=15
2	Sedaxane: 1200 ppm in Diet	n=15	n=15	n=15	n=15	n=15	-
3	Sedaxane: 3600 ppm in Diet	n=15	n=15	n=15	n=15	n=15	n=15
4	Positive Control: 1200 ppm sodium phenobarbital in Diet	n=15	n=15	n=15	n=15	n=15	-

[R] Following 28 days of treatment, 15 animals from the control (Group 1) and 3600 ppm sedaxane (Group 3) groups were retained off-dose for a further 60 days to assess the reversibility of the effects of treatment.

Liver Biochemistry (Microsomal Protein, UGT Activity, Total CYP Content and PROD Activity) in male <u>rats</u>

The treatment of male rats with 1200 ppm sedaxane for 3, 14 and 28 days and 3600 ppm sedaxane for 3, 7, 14 and 28 days resulted in significant increases in hepatic microsomal protein content. Treatment with 1200 and 3600 ppm sedaxane for 1, 3, 7, 14 and 28 days significantly increased hepatic microsomal UDP-glucuronosyltransferase (UGT) activity towards thyroxine as substrate expressed as specific activity, per gram of liver, per total liver and per relative liver weight. The hepatic enzyme induction effects of sedaxane were reversible, as the statistically significant increases in microsomal protein content and UGT activity towards thyroxine as substrate that were observed after 28 days of sedaxane treatment were no longer observed following the recovery period (**Table 4**). Small but statistically significant decreases in UGT activity were observed when enzyme activity was expressed per gram of liver, per total liver and per relative liver weight after the recovery period, but considering the direction of change versus control, these small decreases in hepatic UGT activity are not considered to be of any toxicological significance (data not shown). Treatment with 1200 ppm phenobarbital as a positive control for 3, 7, 14 and 28 days resulted in significant increases in hepatic microsomal protein content and in UGT activity towards thyroxine as substrate expressed as specific activity, per gram of liver, per total liver and per relative liver weight.

The treatment of male rats with 3600 ppm sedaxane and 1200 ppm phenobarbital for 7 days resulted in significant increases in hepatic microsomal total CYP content. Hepatic microsomal 7-pentoxyresorufin O-dependentylase (PROD) activity was significantly increased by treatment with 1200 and 3600 ppm sedaxane and 1200 ppm phenobarbital for 7 days (**Table 5**).

TABLE 4. Liver biochemistry (microsomal protein & UGT activity) in male rats[a]

	Day 1		Day 3		Day 7		Day 14	
	Microsomal Protein (mg/g liver)[b]	**UGT activity (nmol/min /liver wt/kg body wt)**	**Microsomal Protein (mg/g liver)[b]**	**UGT activity (nmol/min /liver wt/kg body wt)**	**Microsomal Protein (mg/g liver)[b]**	**UGT activity (nmol/min /liver wt/kg body wt)**	**Microsomal Protein (mg/g liver)[b]**	**UGT activity (nmol/min /liver wt/kg body wt)**
0	27.8 ± 2.37	9.50 ± 1.320	30.3 ± 2.61	13.20 ± 3.835	28.5 ± 2.84	7.52 ± 1.476	27.6 ± 2.51	7.81 ± 2.236
1200 ppm sedaxane	27.9 ± 4.62 (+0.4%)	12.96 ± 4.017* (+36%)	34.3 ± 3.60* (+13%)	24.94 ± 8.426* (+89%)	30.9 ± 2.50 (+8%)	15.82 ± 3.816* (+110%)	33.8 ± 4.56* (+22%)	16.10 ± 5.982* (+106%)
3600 ppm sedaxane	28.7 ± 4.01 (+3%)	13.83 ± 3.209* (+46%)	34.4 ± 2.60* (+14%)	42.45 ± 13.694* (+222%)	33.1 ± 2.25* (+16%)	33.03 ± 8.242* (+339%)	35.8 ± 3.01* (+30%)	28.96 ± 5.833* (+271%)
1200 ppm positive control (sodium phenobarbital)	25.5 ± 2.71 (-8%)	10.38 ± 2.662 (+9%)	34.3 ± 2.79* (+13%)	27.08 ± 4.616* (+105%)	37.6 ± 5.12* (+32%)	25.06 ± 6.599* (233%)	40.0 ± 3.02* (+45%)	26.30 ± 3.615* (+237%)

	Day 28		Day 88	
	Microsomal Protein (mg/g liver)[b]	**UGT activity (nmol/min /liver wt/kg body wt)**	**Microsomal Protein (mg/g liver)[b]**	**UGT activity (nmol/min /liver wt/kg body wt)**
0	28.3 ± 1.99	6.41 ± 1.484	30.6 ± 2.30	4.39 ± 0.936
1200 ppm sedaxane	31.9 ± 2.02* (+13%)	10.54 ± 3.104* (+64%)	NA	NA
3600 ppm sedaxane	34.6 ± 2.62* (+22%)	25.42 ± 5.883* (+297%)	29.4 ± 2.73 (-4%)	3.62 ± 0.977** (-17%)
1200 ppm positive control (sodium phenobarbital)	37.7 ± 3.04* (+33%)	28.07 ± 6.528* (+338%)	NA	NA

[a] Data obtained from pages 790-801 in the study report (MRID 49804819).

[b] Results are presented as mean ± SD for groups of 15 animals. Values in parentheses are percentage of control levels.

* Statistically different ($p < 0.01$) from the control.

** Statistically different ($p < 0.05$) from the control.

NA= not available

TABLE 5. Liver biochemistry (total CYP content & PROD activity) in male rats[a]

	Day 7	
	Total CYP Content (nmol/mg protein)[b]	**PROD activity (pmol/min/mg protein)[b]**
0	0.35 ± 0.045	15 ± 2.0
1200 ppm sedaxane	0.38 ± 0.039 (+9%)	486 ± 184.0* (+3140%)
3600 ppm sedaxane	0.46 ± 0.078* (+31%)	1035 ± 290.1* (+6800%)
1200 ppm positive control (sodium phenobarbital)	1.04 ± 0.238* (+197%)	1569 ± 290.4* (+10360%)

[a] Data obtained from pages 802 in the study report (MRID 49804819).

[b] Results are presented as mean ± SD for groups of 10 animals. Values in parentheses are percentage of control levels.

* Statistically different ($p < 0.01$) from the control.

CARC concluded that sedaxane treatment leads to an increase in microsomal protein content, UGT activity and PROD activity at 1200 and 3600 ppm, in addition to an increase in total CYP content at 3600 ppm in rats to support Associative Event 1 in the proposed mode of action.

28-day Comparative Isomer Toxicity in rats (*trans*-isomer, *cis*-isomer and a racemic 1:1 *cis-trans* mixture of sedaxane) (Oral-dietary) (MRID 47473372) (Associative Events 1, 2, & 3)

An earlier 28-day comparative isomer toxicity study was conducted in the rat with various ratios of the isomers of sedaxane, to investigate subchronic effects and liver enzyme profiles following 28-day treatment of Wistar rats (Peffer and Noakes, 2010) (MRID 47473372). Groups of five male and five female rats (strain designation: HsdBrlHan:Wistar), from Harlan Labs UK were fed diets containing 0 (control), 500, 2000 or 5000 ppm SYN508210 (*trans*-isomer), SYN508211 (*cis*-isomer) or SYN524464 (1:1 mix of isomers) for 28 consecutive days. The key endpoints of interest to this liver tumor MOA are summarized in **Table 6**.

With all isomer ratios tested, PROD activity (a marker of CYP2b activity) was greatly increased by treatment at 2000 ppm and 5000 ppm, whereas only a small increase in PROD activity was observed at 500 ppm. Testosterone 16β-hydroxylase activity (also a marker of CYP2b activity) showed a similar pattern as PROD, with large increases at 2000 ppm and above.

EROD activity (ethoxyresorufin-O-dealkylase, a marker of CYP1a activity) was virtually unaffected by each isomer, with small increases in activity following SYN508211 treatment that did not display a dose response relationship. Testosterone 6β-hydroxylase activity (a marker of CYP3a activity) was increased somewhat at 2000 ppm and 5000 ppm with all isomers, although the response was variable for SYN508211 and SYN524464. The magnitude of this response indicated weak activity as a CYP3a inducer for the isomers of sedaxane.

Corresponding to these liver enzyme activities, the liver weight adjusted for body weight was significantly increased at 2000 ppm and 5000 ppm, but not at 500 ppm. The only histopathology change observed in the livers was centrilobular hypertrophy. Other liver biochemical endpoints that were assessed in this study (e.g. immunoblots for different CYP proteins, other testosterone hydroxylase activities) were either consistent with the patterns already described in Table 7, or were unaffected. The ratio of isomers in sedaxane technical, SYN508210:SYN508211 (approximately 85:15 ratio), differs from the ratios tested in this study, but the results are informative of the major types of effects in the rat liver with sedaxane isomers.

TABLE 6: Summary of liver data from 28-day subchronic rat study with sedaxane isomers

	SYN508210 (*trans*-isomer)			
	0 ppm	**500 ppm**	**2000 ppm**	**5000 ppm**
Liver Enzymes:				
PROD Activity (pmol/min/mg protein)	4.04	8.40**	258.81**	475.24**
EROD Activity (pmol/min/mg protein)	20.4	22.7	22.1	16.5
Testosterone 6β-hydroxylase Activity (nmol/10 min/mg protein)	5.098	5.720	6.885	9.627**
Testosterone 16β-hydroxylase Activity (nmol/10 min/mg protein)	0.293	0.647	7.086***	10.422***
Weights:				
Terminal Body Wt. (g)[a]	280.6	282.6	285.0	237.6**
Adjusted Liver Wt. (g)	9.4	10.0	11.9**	16.1**
Liver Micropathology (n):	(5)	(5)	(5)	(5)
Centrilobular hypertrophy	0	0	5**	5**
	SYN508211 (*cis*-isomer)			
	0 ppm	**500 ppm**	**2000 ppm**	**5000 ppm**
Liver Enzymes:				
PROD Activity (pmol/min/mg protein)	4.04	22.21**	209.28**	184.77**
EROD Activity (pmol/min/mg protein)	20.4	39.9*	33.3*	28.4
Testosterone 6β-hydroxylase Activity (nmol/10 min/mg protein)	5.098	6.080	11.688***	8.464*
Testosterone 16β-hydroxylase Activity (nmol/10 min/mg protein)	0.293	1.411	5.838***	6.066***
Weights:				
Terminal Body Wt. (g)[a]	280.6	281.4	260.0*	231.8**
Adjusted Liver Wt. (g)	9.4	10.0	12.6**	15.6**
Liver Micropathology (n):	(5)	(5)	(5)	(5)
Centrilobular hypertrophy	0	0	0	5**
	SYN524464 (1:1 isomer ratio)			
	0 ppm	**500 ppm**	**2000 ppm**	**5000 ppm**
Liver Enzymes:				
PROD Activity (pmol/min/mg protein)	4.04	14.06**	294.53**	201.28**
EROD Activity (pmol/min/mg protein)	20.4	26.7	30.7	20.9
Testosterone 6β-hydroxylase (nmol/10 min/mg protein)	5.098	6.297	11.440***	6.986

Testosterone 16β-hydroxylase (nmol/10 min/mg protein)	0.293	1.274	6.835***	6.557***
Weights:				
Terminal Body Wt. (g)[a]	280.6	296.8	265.6	238.4**
Adjusted Liver Wt. (g)	9.4	9.8	12.6**	16.5**
Liver Micropathology (n):	(5)	(5)	(5)	(5)
Centrilobular hypertrophy	0	0	5**	5**

*, **, *** Statistically-significantly different from control with $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

[a] Terminal body weight was statistically analyzed after adjustment for initial Day 1 weight. Data from Peffer & Noakes (2010).

CARC concluded that sedaxane treatment leads to an increase in PROD activity (a marker of CYP2b activity; 500, 2000 and 5000 ppm), testosterone 16β-hydroxylase activity (also a marker of CYP2b activity; 2000 and 5000 ppm), and testosterone 6β-hydroxylase activity (a marker of CYP3a activity; 2000 and 5000 ppm but varied between isomers) (Associative Event 1). In addition, there was an increase in liver weight adjusted for body weight (Associative Event 3) and an increase in centrilobular hypertrophy (Associative Event 2) (2000 and 5000 ppm but varied between isomers) in rats for the mode of action hypothesis presented in Figure 1.

c) Altered gene expression and liver enzyme activity in sedaxane-treated male mice (Associative Event 1 and Key Events 2 & 3)

A 21 Day Dietary Liver Mode of Action Study in Male CD-1 Mice (MRID 49804810)

Male CD-1 mice [strain designation Crl:CD-1 (ICR), 6 mice/group/time point] were treated with sedaxane at dietary inclusion levels of 0, 1250, 7000 and 14000 ppm (equivalent to 0, 170, 944 and 1792 mg/kg/day) for 1, 3, 7 or 21 days before termination (Study Days 2, 4, 8 and 22) (MRID 49804810). The 1250 ppm dose was the mid-dose in the 18- month carcinogenicity study, 7000 ppm was the highest dose in the carcinogenicity study and the only dose where a statistically significantly higher incidence of combined (adenoma + carcinoma) liver tumors was recorded, and 14000 ppm was used as a higher dose to explore dose-response effects.

The mouse CAR activator 1,4-bis [2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP) was used as a positive control. The study design was as follows:

Group Number	Treatment	Number of Male Mice			
		Termination Timepoints (Study Day)			
		2	4	8	22
1	Control: Basal (untreated) Diet	n=6	n=6	n=6	n=6
2	Sedaxane: 1250 ppm in Diet	n=6	n=6	n=6	n=6
3	Sedaxane: 7000 ppm in Diet	n=6	n=6	n=6	n=6
4	Sedaxane: 14000 ppm in Diet	n=6	n=6	n=6	n=6
5	Positive Control: TCPOBOP in DMSO	n=6 [1]	n=6 [2]	-	-

6	DMSO Vehicle Control	n=6 [1]	n=6 [2]	-	-

[1] The first six mice in Groups 5 and 6 were euthanized approximately 12 hours after a single intraperitoneal (ip) 3 mg/kg dose of TCPOBOP (or vehicle).

[2] The last six mice in Groups 5 and 6 were dosed twice with TCPOBOP at 3 mg/kg/dose (or vehicle), appropriately 48 hours between doses, and euthanized approximately 12 hours after the second dose.

Toxicogenomics (RT-PCR) and liver biochemistry in male mice

Select liver mRNA transcript levels were measured in male mice following sedaxane treatment. Based on analysis of liver mRNA expression by RT-PCR, the treatment of male mice with 1250, 7000 or 14000 ppm sedaxane resulted in a significant dose-dependent up-regulation of Cyp2b10 mRNA levels at all dose levels and time points. With the exception of the effect of treatment with 1250 ppm for 21 days, a significant dose-dependent up-regulation of Cyp2c65 mRNA levels was also observed at all dose levels and time points. The treatment of male mice with 14000 ppm significantly up-regulated hepatic Gadd45β mRNA levels on Days 2, 4 and 8, and Gadd45β mRNA levels also were significantly up-regulated after treatment with 7000 ppm on Day 2. A large mean value for Gadd45β mRNA in the 7000 ppm treated animals was also observed on Day 4, but the effect was not statistically significant when one outlier animal was excluded from the analysis. Sedaxane had no treatment-related effects on hepatic Cdc20 mRNA levels or Fos mRNA levels at any time point. Statistical differences in Fos mRNA on Day 4 were attributable to low control group ΔCt values at this time point, rather than to any effect of sedaxane.

With Day 8 liver samples, treatment of male mice with 1250, 7000 or 14000 ppm sedaxane produced a significant dose-dependent induction of 7-pentoxyresorufin O-depentylase (PROD) activity (a marker of Cyp2b activity), and treatment with 14000 ppm also produced a significant increase in testosterone 6β-hydroxylase activity (marker of Cyp3a activity). This pattern is consistent with the mouse CAR and PXR activation assays (MRID 49804825 and MRID 49804824), which showed that sedaxane was a CAR activator but not a PXR activator for the mouse nuclear receptor. There is cross-talk between CAR and PXR and some overlap in the extent of the activation of these Cyp isoforms, which could be responsible for the small increase in Cyp3a activity.

Treatment with a single 3 mg/kg intraperitoneal dose of TCPOBOP resulted in significant increases in hepatic Cyp2b10, Cyp2c65 and Gadd45β mRNA levels, whereas treatment with two 3 mg/kg intraperitoneal doses of TCPOBOP resulted in significant increases in hepatic Cyp2b10, Cyp2c65, Gadd45β and Cdc20 mRNA levels. While an up-regulation of Fos mRNA levels on Day 4 was also observed, this effect was not statistically significant when one animal considered to be an outlier was excluded from the analysis. A summary of fold changes for hepatic mRNA levels is presented in **Table 7**. Hepatic microsomal protein content, PROD levels and testosterone 6β-hydroxylase activities are presented in **Table 8**.

TABLE 7. Summary fold change data and statistics for hepatic mRNA levels in male mice [a,b,c]

Sacrifice	Group/Concentration	Hepatic mRNA- Fold Induction				
		Cyp2b10	Cyp2c65	Gadd45β	Cdc20	Fos

Day 2	Group 2/ 1250 ppm sedaxane	44.2**	4.5**	2.5	1.4	1.3
	Group 3/ 7000 ppm sedaxane	163.0**	13.2**	12.5**	2.2	1.3
	Group 4/ 14000 ppm sedaxane	231.1**	19.6**	38.8**	2.0	2.9
	DMSO Control	1.0	1.0	1.0	1.0	1.0
	TCPPOBOP/ 3 mg/kg	1436.8**	11.0**	35.5**	0.9	0.8
Day 4	Group 1/ 0 ppm sedaxane	1.0	1.0	1.0	1.0	1.0
	Group 2/ 1250 ppm sedaxane	61.7**	4.3**	3.9	0.9	0.2**
	Group 3/ 7000 ppm sedaxane	197.1**	20.2**	20.5* (3.2)	1.0	0.1**
	Group 4/ 14000 ppm sedaxane	294.7**	49.0**	7.0* (7.0)**	1.5	0.1**
	DMSO Control	1.0	1.0	1.0	1.0	1.0
	TCPPOBOP/ 3 mg/kg	62.4**	68.9**	20.9**	56.4** (67.0)**	16.3* (4.8)
Day 8	Group 1/ 0 ppm sedaxane	1.0	1.0	1.0	1.0	1.0
	Group 2/ 1250 ppm sedaxane	15.4**	2.4**	1.1	1.3	0.9
	Group 3/ 7000 ppm sedaxane	52.0**	14.8**	1.7	1.5	0.7
	Group 4/ 14000 ppm sedaxane	98.0**	49.1**	4.1**	1.1	0.7
Day 22	Group 1/ 0 ppm sedaxane	1.0	1.0	1.0	1.0	1.0
	Group 2/ 1250 ppm sedaxane	4.4**	1.3	0.9	1.0	1.1
	Group 3/ 7000 ppm sedaxane	9.6**	13.5**	0.7	1.5	1.9
	Group 4/ 14000 ppm sedaxane	19.0**	67.0**	0.9	1.3	1.6

[a] Taken from study report page 276 (MRID 49804810).
[b] Results are presented as mean values (rounded to nearest 0.1) for groups of 6 animals. Values in parentheses are mean values of 5 animals excluding outliers for 3 days of treatment. Theses outliers comprised one animal in Group 3 for Gadd45β mRNA levels and one animal each in Group 5 for Cdc20 and Fos mRNA levels.
[C] Fold changes in gene expression are calculated as 2 to the power of the $-\Delta\Delta Ct$ value. Statistical analysis of data was performed on the ΔCt values
Values significantly different from controls are: *p<0.05; **p<0.01

Table 8: Hepatic microsomal protein content and 7-pentoxyresorufin O-dependentylase (PROD) and testosterone 6β -hydroxylase activities in male mice

Treatment[a]	Microsomal protein (mg/g liver)	PROD (pmol/min/mg protein)	Testosterone 6β-hydroxylase (nmol/min/mg protein)
Control	30.3 ± 2.25[b] (100)	18 ± 6.2 (100)	0.98 ± 0.263 (100)
Sedaxane 1250 ppm	32.6 ± 2.53 (108)	50 ± 12.3** (278)	0.96 ± 0.195 (98)
Sedaxane 7000 ppm	32.1 ± 1.97 (106)	266 ± 45.5** (1478)	1.23 ± 0.158 (126)
Sedaxane 14000 ppm	31.3 ± 2.22 (103)	439 ± 84.6** (2439)	2.55 ± 0.551** (260)

[a] Animals were fed control diet or diet containing sedaxane for 7 days.

[b] Results are presented as mean ± SD for groups of 6 animals. Values in parentheses are percentage of control levels. Values significantly different from control are: **$p<0.01$.
MRID 49804810

Liver toxicogenomics (microarray analysis) in male mice

Liver microarrays were evaluated for changes in overall pathways (by IPA analysis) and for significant changes in families of related mRNAs for the sedaxane-treated groups on Days 2, 4 and 22. There were only minimal differences between control and 1250 ppm groups in the number of differentially expressed genes (DEGs), whereas progressively greater numbers of DEGs were observed with 7000 ppm and 14000 ppm sedaxane treatment. Pathway analysis revealed changes involved in xenobiotic metabolism at 7000 ppm and 14000 ppm on Days 2, 4, and 22. The changes involving *Cyp2b10* (maximal at Day 4, 14000 ppm, *ca.*126 fold upregulated) and *Cyp3a11* (maximal at Day 2, 14000 ppm, *ca.*15 fold up-regulated) indicate the involvement of the nuclear receptors CAR and PXR, respectively. However, cross-talk between CAR and PXR and overlap in the genes that these nuclear receptors activate is known to occur (Elcombe et al., 2014).

Data from canonical pathway analysis by IPA also suggests involvement of these nuclear receptors. Unbiased pathway analyses of the microarray results from Genespring using IPA were in concordance with those of Oshida et.al. (2015), where the top 10 IPA pathways in mouse liver following treatment with model CAR activators were virtually identical to those identified for sedaxane.

A lack of *Cyp4a* induction coupled with minimal PPAR-α pathway gene induction suggests no significant involvement of peroxisome proliferator-activated receptor alpha (PPAR-α). In the absence of *Cyp1a1* induction there is no strong evidence for Aryl hydrocarbon receptor (AhR) activation. The few changes observed related to AhR were relatively small in number and were not definitive of AhR activation due to the fact that they are also regulated by other nuclear hormone receptors.

Changes in genes involved in cell cycle including up-regulation of *Gasdd45β* (14000 ppm at Days 2, 4 and 22) and down-regulation of *Gadd45gamma* (7000 ppm at Day 22) were noted only at higher dose levels. The polarity and magnitude of fold change may be consistent with a weak/mild proliferative effect in the liver at these dose levels.

CARC concluded, based on combined data from RT-PCR and microarray analysis, that the increased expression of hepatic Cyp2b10 mRNA, Cyp2c65 mRNA, and PROD activity at 1250, 7000, and 14000 ppm is consistent with Associative Event 1. In addition, there was also an increase in Gadd45β mRNA at 7000 and 14000 ppm and testosterone 6β-hydroxylase activity at 14000 ppm. The molecular data were also supported by microarray data showing increases in expression of xenobiotic metabolizing enzymes and other genes associated with CAR/PXR activation at 7000 and 14000 ppm in mice (Key Events 2 and 3 for the mode of action hypothesis presented in Figure 1).

d) Cell proliferation in sedaxane-treated male rats (Key Event 4)

28 Day Oral (Dietary) Mechanistic Study to Evaluate Effects on the Liver and Thyroid on the Male Rat (MRID 4984819)

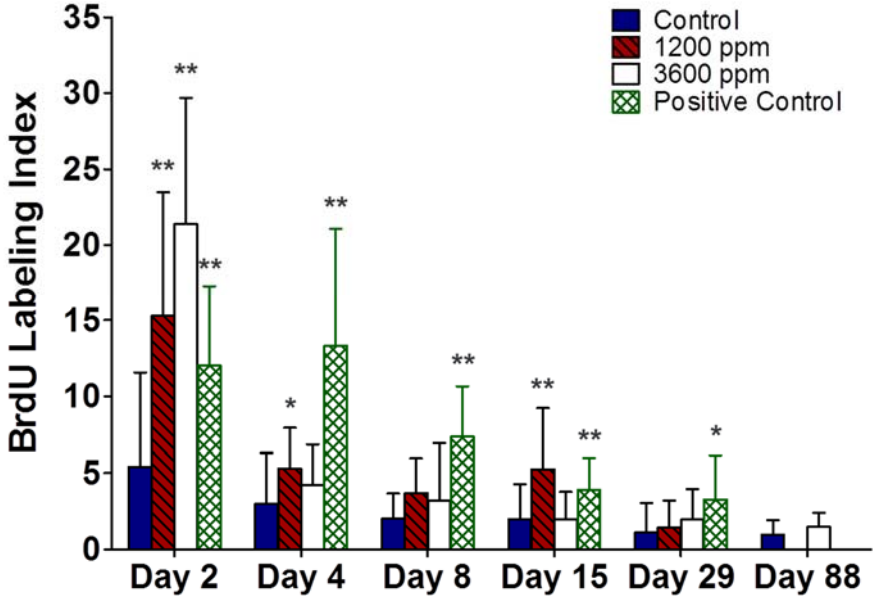
The design for the 28-day dietary study in male rats is previously described (Section III C1. b). All animals in this study received a subcutaneous injection of BrdU in sterile 0.9% sodium chloride solution approximately two hours before termination. The formalin-fixed paraffin-embedded liver samples of all rats were processed by immunohistochemistry to detect and quantify the labeling-index (% positive BrdU hepatocytes) as a measurement of S-phase or cell proliferation. A statistically significant transient increase in hepatocellular proliferation, as indicated by an elevated hepatic BrdU labeling index was evident after two days of treatment with sedaxane at both 1200 and 3600 ppm. Proliferation, as measured by the labeling index, was less evident on day four of treatment (with only the 1200 ppm sedaxane treatment achieving statistical significance) and by day 29 of treatment, the labeling index in all the sedaxane treatment groups returned to baseline levels which was maintained through day 89. Labeling indices of the positive control increased in a manner consistent with the known toxicity of the test item. BrdU group mean cell counts are presented in **Table 9** and graphed in **Figure 3**.

TABLE 9. BrdU cell count group mean values in male rats [a]

Dose rate ppm	Cell Count (BrdU/1000) Mean ± SD					
	Day 2	Day 4	Day 8	Day 15	Day 29	Day 89
0	5.426 ± 6.144	3.006 ± 3.321	2.006 ± 1.683	1.994 ± 2.289	1.136 ± 1.914	0.972 ± 0.967
1200 ppm sedaxane	15.272 ± 8.214*	5.297 ± 2.663**	3.695 ± 2.271	5.265 ± 3.966*	1.369 ± 1.791	NA
3600 ppm sedaxane	21.387 ± 8.301*	4.210 ± 2.652	3.231 ± 3.711	1.993 ± 1.782	1.963 ± 2.001	1.473 ± 0.939
1200 ppm positive control (sodium phenobarbital)	12.048 ± 5.251*	13.342 ± 7.719*	7.365 ± 3.300*	3.903 ± 2.091*	3.276 ± 2.889**	NA

[a] Data obtained from pages 76-86 in the study report (MRID 4984819).
* Statistically different ($p < 0.01$) from the control (Dunnett 2-sided test).
** Statistically different ($p < 0.05$) from the control (Dunnett 2-sided test).
NA= data not available

Figure 3: Cell Proliferation Results by BrdU Labeling Index in Rat Livers



*, ** Statistically-significantly different from control with p<0.05 and p<0.01, respectively. Data from MRID 498481.

CARC concluded that sedaxane treatment leads to a transient increase in hepatocellular proliferation in rats as measured by the BrdU labeling index at 1200 and 3600 ppm (Key Event 4 for the mode of action hypothesis presented in Figure 1).

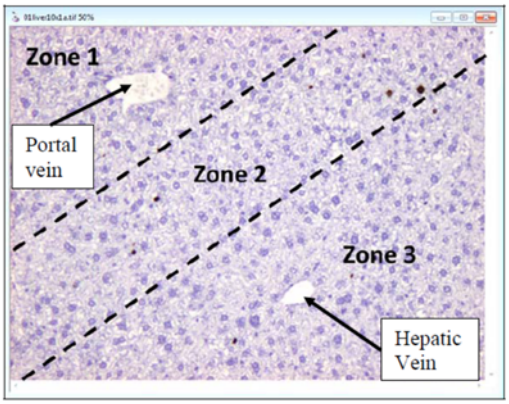
e) Cell proliferation in sedaxane-treated male mice (Key Event 4)

A 21 Day Dietary Liver Mode of Action Study in Male CD-1 Mice (MRID 49804810)

Cell proliferation by Ki67 with zonal counts

The design for the 21-day dietary study in male mice is previously described (Section III C1. c). In the 21-day MOA study in male mice, cell proliferation was evaluated by the zonal distribution of Ki67 in the liver, as illustrated in **Figure 4**. Zone 1 corresponded to the periportal region (including the portal triad), zone 2 was the midzonal region and zone 3 corresponded to the centrilobular region (including the hepatic vein). Ki67 is a nuclear protein that is expressed during all phases of the proliferating cell cycle (G1, S and G2), but not during the quiescent G0 phase. In contrast, BrdU is incorporated into newly synthesized DNA only during S-phase of the cell cycle.

Figure 4: Liver Zone Designations- Ki67 Analysis of Mouse Livers



The Ki67 immunostaining (brown chromogenic labeling with DAB) was appropriately restricted to the nuclei of hepatocytes and occasionally other cell types (e.g., Kupffer cells). The image analysis software was able to distinguish between these cell types *via* size and shape criteria. A slight treatment-related effect was observed at the Day 8 sacrifice, as indicated by statistically significant increases relative to controls in mean labeling index (LI) values for zones 1, 2, and 3 combined in the 7000 ppm ($p = 0.0386$) and 14000 ppm ($p = 0.0097$) dose groups as determined by the student's t-test analysis. These results also approached significance using the Dunnett's test (with p-values of 0.0604 and 0.0569, respectively). The overall ANOVA analysis for all dose groups and combined zones at Day 8 was additionally significant at $p = 0.0186$. These significant results for the combined zones were driven primarily by mean LI increases in zone 3 (7000 and 14000 ppm) and zone 1 (14000 ppm); no significant changes in LI in zone 2 were noted at any time point or sedaxane dietary treatment level. The only other significant results were decreases relative to controls in the combined zone ($p = 0.0011$) and zone 1 ($p = 0.0479$) mean LI values for the 1250 ppm dose group at the Day 22 sacrifice based on the student's t-test. However, there were no comparable mean LI decreases in the two higher (i.e., 7000 and 14000 ppm) dose groups, and analysis results for the 1250 ppm dose group did not approach significance in either the Dunnett's or ANOVA tests; thus it is unlikely that these single group decreases in mean LI are toxicologically meaningful. The liver Ki67 labeling index results are presented in **Table 10** and graphed in **Figure 5**.

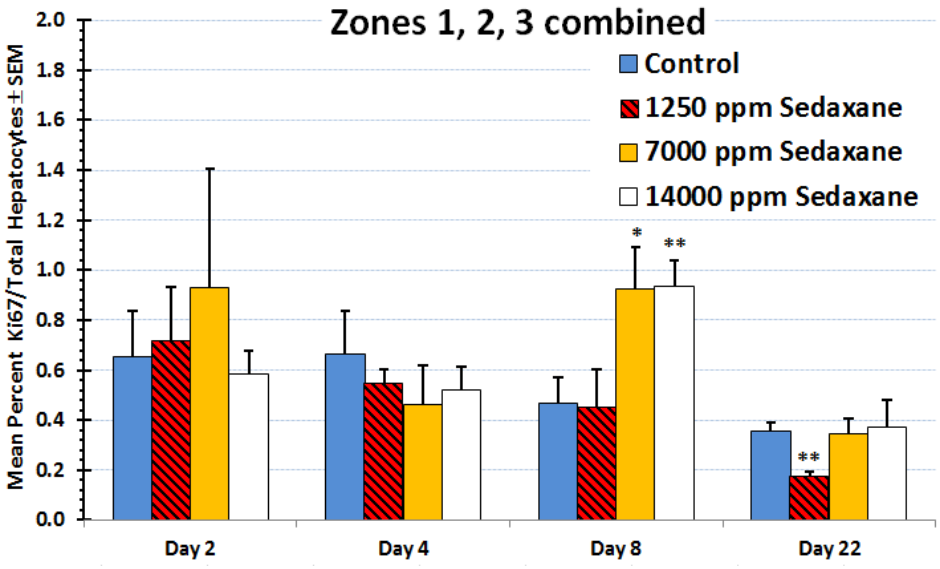
Table 10: Liver Ki67 labeling index results in male mice [a]

Sacrifice	Group/Concentration	Liver Zones (mean ± SD)			
		1, 2 and 3 combined	1	2	3
Day 2	Group 1/ 0 ppm sedaxane	0.65637 ± 0.43807	1.01975 ± 0.83905	0.53375 ± 0.39105	0.43201 ± 0.21342
	Group 2/ 1250 ppm sedaxane	0.71701 ± 0.53504	0.92983 ± 0.52264	0.65316 ± 0.59174	0.57030 ± 0.55594
	Group 3/ 7000 ppm sedaxane	0.92870 ± 1.17228	1.06214 ± 1.22087	0.72700 ± 1.11808	0.99212 ± 1.24079
	Group 4/ 14000 ppm sedaxane	0.58731 ± 0.22803	0.8634 ± 0.42364	0.48795 ± 0.41315	0.42096 ± 0.12906
Day 4	Group 1/ 0 ppm sedaxane	0.66599 ± 0.41973	0.91316 ± 0.67856	0.64519 ± 0.35259	0.38318 ± 0.30072

	Group 2/ 1250 ppm sedaxane	0.54734 ± 0.13926	0.71395 ± 0.26568	0.47667 ± 0.27158	0.44569 ± 0.13413
	Group 3/ 7000 ppm sedaxane	0.46424 ± 0.37552	0.66193 ± 0.48612	0.37713 ± 0.36699	0.35894 ± 0.42791
	Group 4/ 14000 ppm sedaxane	0.5211 ± 0.2244	0.61591 ± 0.23468	0.65172 ± 0.42741	0.32181 ± 0.31035
Day 8	Group 1/ 0 ppm sedaxane	0.46856 ± 0.24984	0.47894 ± 0.4873	0.45041 ± 0.30013	0.47363 ± 0.24963
	Group 2/ 1250 ppm sedaxane	0.45151 ± 0.36586	0.71786 ± 0.4534	0.28342 ± 0.2865	0.34462 ± 0.4621
	Group 3/ 7000 ppm sedaxane	**0.92718* ± 0.40026**	0.96521 ± 0.78651	0.87619 ± 0.43332	**0.97014* ± 0.30893**
	Group 4/ 14000 ppm sedaxane	**0.93277** ± 0.25484**	**1.04726* ± 0.33408**	0.84844 ± 0.3818	**0.89879* ± 0.2663**
Day 22	Group 1/ 0 ppm sedaxane	0.35519 ± 0.0827	0.38425 ± 0.13893	0.2367 ± 0.18065	0.44281 ± 0.27891
	Group 2/ 1250 ppm sedaxane	**0.17224** ± 0.05527**	**0.20614* ± 0.13477**	0.11701 ± 0.07824	0.19333 ± 0.13787
	Group 3/ 7000 ppm sedaxane	0.34607 ± 0.14801	0.54235 ± 0.22655	0.21428 ± 0.13364	0.26281 ± 0.17594
	Group 4/ 14000 ppm sedaxane	0.37407 ± 0.26658	0.53668 ± 0.37862	0.27635 ± 0.25434	0.30852 ± 0.22136

[a] Taken from study report page 314 (MRID 49804810)
n=6
*Significance level of comparison with Group 1, $p \leq 0.05$, student's t-test
**Significance level of comparison with Group 1, $p \leq 0.01$, student's t-test

Figure 5: Results of Ki67 Cell Proliferation Analysis of Livers- Sedaxane-Treated Mice



*, ** Statistically-significantly different from control with $p<0.05$ and $p<0.01$, respectively. Data from MRID 49804810.

Cell proliferation by BrdU

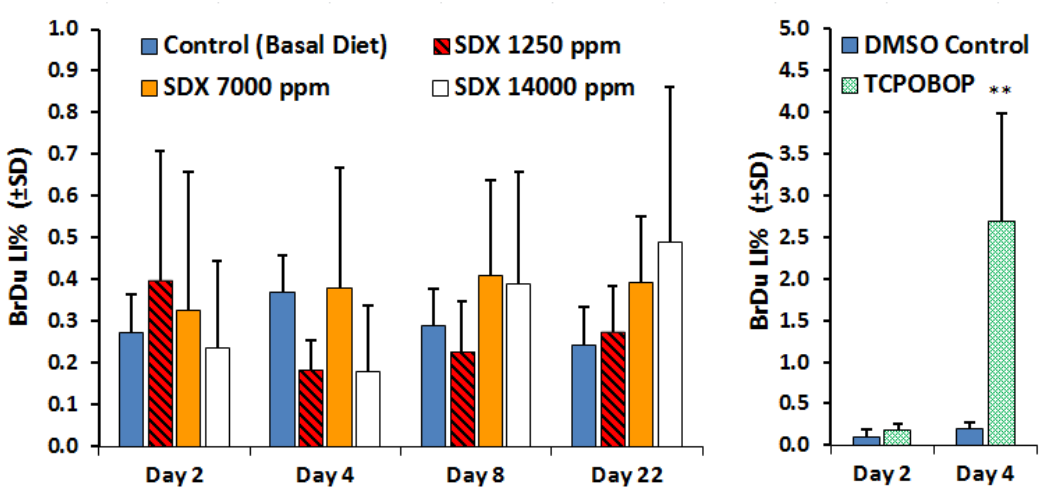
The treatment of male mice with sedaxane in their diets did not cause a statistically significant increase in BrdU labeling index at any time point. However, there was a numerical trend toward higher mean values in the 7000 ppm and 14000 ppm sedaxane groups on Days 8 and 22 of treatment (1.3 – 2.0-fold) compared to the time-matched controls. However, due to high inter-animal variability in the labeling index values, the mean values were not statistically significant. The positive control agent TCPOBOP produced a 14-fold increase in BrdU labeling index ($p<0.01$) after two treatments at 3 mg/kg/day ip (Day 4), but it had no effect after one treatment (Day 2). Liver BrdU labeling index results are presented in **Table 11** and graphed in **Figure 6**.

TABLE 11: Liver BrdU labeling index results in male mice [a]

Group No.	Treatment	BrdU Labeling Index (mean LI% ± SD)			
		Sacrifice Timepoints			
		Day 2	Day 4	Day 8	Day 22
1	Control Basal Diet	0.27 ± 0.17	0.37 ± 0.20	0.29 ± 0.13	0.24 ± 0.09
2	1250 ppm Sedaxane	0.40 ± 0.31	0.18 ± 0.07	0.23 ± 0.12	0.27 ± 0.11
3	7000 ppm Sedaxane	0.33 ± 0.33	0.38 ± 0.29	0.41 ± 0.23	0.39 ± 0.16
4	14000 ppm Sedaxane	0.24 ± 0.21	0.18 ± 0.16	0.39 ± 0.27	0.49 ± 0.37
5	TCPOBOP	0.17 ± 0.09	2.68 ± 1.30*	NA	NA
6	DMSO Vehicle	0.10 ± 0.06	0.19 ± 0.08	NA	NA

[a] Data obtained from page 246 in the study report (MRID 49804810).
* Statistically different from the control student's t-test, $p<0.01$
NA= data not available

Figure 6: CD-1 Mouse Liver BrdU Labeling Index Results



Results are means ±SD, n=6.
**p<0.01, Student's t-test Data from MRID 49804810.

CARC concluded, based on combined data from Ki67 and BrdU immunohistochemistry of the liver, that a slight, treatment-related increase in cell proliferation was observed on Day 8 following treatment with 7000 and 14000 ppm sedaxane in <u>mice</u> (Key Event 4 for the mode of action hypothesis presented in Figure 1).

f) Liver weight and histopathology in sedaxane-treated male <u>rats</u> (Associative Event 2 & 3)

28 Day Oral (Dietary) Mechanistic Study to Evaluate Effects on the Liver and Thyroid on the Male Rat (MRID 4984819)

The design for the 28-day dietary study in male rats was previously described (Section III C1. b). Liver weights (absolute, adjusted for body weight and expressed as a percentage of body weight), were notably elevated from day 4 onwards for animals given 1200 or 3600 ppm sedaxane (**Table 12**). Where statistical analysis was applied (absolute and adjusted for body weight), these differences were significant ($p<0.05$ or $p<0.01$) when compared with controls. At the end of the treatment-free period, liver weights in animals previously treated at 3600 ppm sedaxane were similar to controls indicating the liver weight increases were fully reversible. In animals given 1200 ppm sodium phenobarbital (NaPB; positive control), absolute liver weights were elevated on day 2 and both absolute and body weight adjusted liver weights were significantly increased from day 4 onward ($p<0.05$ - $p<0.01$). This group showed elevated thyroid weights on days 2, 15 and 29 but not on days 4 or 8.

TABLE 12: Liver weights (Mean ± SD) in male rats [a]

	Day 2			Day 4			Day 8		
	Liver (g)	**Adjusted (g)**	**% Body Weight**	**Liver (g)**	**Adjusted (g)**	**% Body Weight**	**Liver (g)**	**Adjusted (g)**	**% Body Weight**
0	8.82 ± 0.66	8.62	4.46 ± 0.19	9.75 ± 1.12	9.62	4.18 ± 0.25	9.91 ± 0.78	9.46	4.20 ± 0.27
1200 ppm sedaxane	9.05 ± 1.18	8.87	4.58 ± 0.36	10.80 ± 1.49**	10.52* (+9%)	4.57 ± 0.27	11.43 ± 1.85*	11.28* (+19%)	4.92 ± 0.37
3600 ppm sedaxane	8.24 ± 1.29	8.61	4.38 ± 0.35	11.29 ± 1.12*	11.70* (+22%)	5.08 ± 0.26	12.42 ± 0.99*	13.02* (+38%)	5.69 ± 0.42
1200 ppm positive control (sodium phenobar bital)	9.33 ± 0.70**	9.18	4.54 ± 0.24	12.31 ± 0.91*	11.98* (+25%)	5.00 ± 0.25	12.66 ± 1.25*	12.55* (+33%)	5.27 ± 0.37

[a] Data obtained from pages 88-98 in the study report (MRID 4984819).
* Statistically different ($p < 0.01$) from the control (Dunnett 2-sided test).
** Statistically different ($p < 0.05$) from the control (Dunnett 2-sided test).
NA= data not available.
Percent changes are presented in parentheses and were calculated by the reviewer.

TABLE 12 (continued): Liver weights (Mean ± SD) in male rats [a]

	Day 15			Day 29			Day 89		
	Liver (g)	**Adjusted (g)**	**% Body Weight**	**Liver (g)**	**Adjusted (g)**	**% Body Weight**	**Liver (g)**	**Adjusted (g)**	**% Body Weight**
0	10.68 ± 1.08	10.43	3.88 ± 0.19	10.48 ± 1.60	10.15	3.51 ± 0.26	11.90 ± 0.93	11.56	3.05 ± 0.19
1200 ppm sedaxane	12.53 ± 1.79*	12.06* (+16%)	4.46 ± 0.28	11.88 ± 1.32**	11.48* (+13%)	3.97 ± 0.19	NA	NA	NA
3600 ppm sedaxane	13.12 ± 1.33*	13.86* (+33%)	5.18 ± 0.33	12.80 ± 1.49*	13.53* (+33%)	4.69 ± 0.23	11.16 ± 1.66	11.49	3.01 ± 0.22
1200 ppm positive control (sodium phenobar bital)	14.05 ± 1.42*	14.02* (+34%)	5.07 ± 0.26	14.56 ± 2.04*	14.17* (+40%)	4.63 ± 0.30	NA	NA	NA

[a] Data obtained from pages 88-98 in the study report (MRID 4984819).
* Statistically different ($p < 0.01$) from the control (Dunnett 2-sided test).
** Statistically different ($p < 0.05$) from the control (Dunnett 2-sided test).
NA= data not available

There were no treatment-related macroscopic findings in liver from animals given 1200 or 3600 ppm sedaxane or for animals given 1200 ppm sodium phenobarbital. The histopathology evaluation showed that treatment with sedaxane at 1200 and 3600 ppm produced a dose- and time-dependent increase in centrilobular hypertrophy (**Table 13**), which correlates with increases in liver weight adjusted for body weight. These changes were not observed on Day 2, but they were present beginning on Day 4 and continued throughout the dosing period. The incidence and

severity of the hypertrophy of hepatocytes was greater at 3600 ppm (minimal to moderate severity) than at 1200 ppm sedaxane (minimal to slight severity). NaPB, the positive control agent, produced similar increases in liver weight and histopathology findings as sedaxane. After the 60-day recovery period (Day 89), no differences from control were observed in the liver for animals that had previously been treated with 3600 ppm sedaxane.

TABLE 13: Liver histopathology from 28-day rat MOA study.

	Sedaxane			NaPB
	0 ppm	**1200 ppm**	**3600 ppm**	**1200 ppm**
Liver: Centrilobular Hypertrophy (N)	(15)	(15)	(15)	(15)
Day 2	0	0	0	0
Day 4	0	7** (minimal)	12** (minimal)	15** (12 minimal/3 slight)
Day 8	0	6* (minimal)	10** (7 minimal/3 slight)	13** (5 minimal/8 slight)
Day 15	0	11** (9 min/2 slight)	13** (5 min/8 slight)	15** (11 slight/4 moderate)
Day 29	0	12** (8 minimal/4 slight)	14** (2 minimal/10 slight/2 moderate)	15** (1 minimal/9 slight/5 moderate)
Day 29 (+60)	0	NA	0	NA

*, **Statistically significant difference from control group incidence by Fisher's Exact Test ($p<0.05$, $p<0.01$) Values shown are total incidence (combining all severities).
NA – Not applicable
Data from Chubb (2015).

CARC concluded that sedaxane treatment leads to an increase in liver weight (Associative Event 3) and hepatocellular hypertrophy (Associative Event 2) in the <u>rat</u> at 1200 and 3600 ppm for the mode of action hypothesis presented in Figure 1.

g) Liver weight and histopathology in sedaxane-treated male <u>mice</u> (Associative Events 2 & 3)

1. <u>A 21 Day Dietary Liver Mode of Action Study in Male CD-1 Mice (MRID 49804810)</u>

The design for the 21-day dietary study in male mice was previously described (Section III C1. C). Statistically significant increases in mean adjusted liver weights were noted for the 14000 ppm sedaxane-treated mice terminated on Day 4, and for the 7000 ppm and 14000 ppm sedaxane-treated mice terminated on Days 8 and 22, as compared to the mean adjusted liver weights of controls. In addition, the mean absolute liver weights for mice terminated on Days 8 and 22 were statistically significantly higher than the control values for the 14000 ppm group. There were no effects on absolute or adjusted liver weight for the 1250 ppm group. The mean adjusted liver weights for the positive control (TCPOBOP) mice euthanized on Days 2 and 4 were statistically significantly higher than those of the DMSO vehicle controls. In addition, the

absolute liver weights for the TCPOBOP treated mice were statistically significantly higher than the controls on Day 4. **Table 14** presented absolute and body weight covariate adjusted liver weights.

TABLE 14. Liver weight parameters in male mice [a]

Group No.	Treatment		Mean Liver Weights (g) (% change from control)			
			Sacrifice Timepoints			
			Day 2	Day 4	Day 8	Day 22
1	Control Basal Diet	Absolute:	1.99	2.22	2.01	1.79
		Adjusted:	1.98	2.18	2.02	1.78
2	1250 ppm Sedaxane	Absolute:	2.09 (+5)	2.20 (-1)	2.11 (+5)	1.86 (+4)
		Adjusted:	2.06 (+4)	2.20 (+1)	2.07 (+2)	1.85 (+4)
3	7000 ppm Sedaxane	Absolute:	1.88 (-6)	2.31 (+4)	2.30 (+14)	1.98 (+11)
		Adjusted:	1.87 (-6)	2.34 (+7)	2.33 (+15) *	1.99 (+12) *
4	14000 ppm Sedaxane	Absolute:	1.88 (-6)	2.60 (+17)	2.62 (+30) *	2.35 (+31) *
		Adjusted:	1.91 (-4)	2.61 (+20) *	2.60 (+29) *	2.36 (+33) *
5	TCPOBOP	Absolute:	2.30 (+7)	2.95 (+31) *	NA	NA
		Adjusted:	2.29 (+7) *	2.95 (+31) *	NA	NA
6	DMSO Vehicle	Absolute:	2.14	2.25	NA	NA
		Adjusted:	2.15	2.25	NA	NA

[a] Data obtained from pages 49-50 in the study report (MRID 49804810).
* Statistically different from the control.
NA= not available

No gross findings were noted in any mouse that received sedaxane. Macroscopic abnormalities were restricted to prominent lobular architecture of the liver, which was observed in one animal that received 3 mg/kg of TCPOBOP and was terminated on Day 4. This finding correlated with centrilobular hepatocyte hypertrophy.

Microscopic pathology showed hepatocyte hypertrophy (centrilobular or diffuse) in the livers of mice treated with sedaxane or with TCPOBOP was apparent beginning on Day 2, and it increased in incidence over time. An increased incidence of hepatocyte hypertrophy (mild severity) was observed in the 14000 ppm treated animals on Days 2 and 4, and in the 7000 and 14000 ppm animals on Days 8 and 22. There were no treatment-related histological findings in the liver for the 1250 ppm group. Test substance-related microscopic findings are summarized in **Table 15**.

TABLE 15. Incidence and severity of treatment-related microscopic findings in the liver in male mice [a]

Group No.	Treatment	Incidence of Hepatocyte Hypertrophy												Inflammatory Cell Foci			
		Centrilobular (mild)				Diffuse (mild)				Centrilobular & Diffuse (mild) Combined							
		Day 2	Day 4	Day 8	Day 22	Day 2	Day 4	Day 8	Day 22	Day 2	Day 4	Day 8	Day 22	Day 2	Day 4	Day 8	Day 22
1	Control Basal Diet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	1250 ppm Sedaxane	0	0	1 (min)	0	0	0	0	0	0	0	1 (min)	0	0	0	0	0
3	7000 ppm Sedaxane	0	0	4	5*	0	0	2	0	0	0	6	5	1	0	0	1
4	14000 ppm Sedaxane	2	3	0	6**	0	1	6**	0	2	4	6	6	0	0	0	0
5	TCPOBOP	4	4	NA		0	0	NA		4	4	NA		1	0	NA	
6	DMSO Vehicle	0	0			0	0			0	0			0	0		

[a] Taken from study report page 50 (MRID 49804810).
* Statistically different from the control at $p<0.05$.
** Statistically different from the control at $p<0.01$.
Statistical significance was not evaluated for combined centrilobular and diffuse hepatocyte hypertrophy.
n= 6 mice/group/day.
NA= data not available.

CARC concluded that sedaxane treatment leads to an increase in liver weight (Associative Event 3) and hepatocellular hypertrophy (Associative Event 2) in mice at 7000 and 14000 ppm for the mode of action hypothesis presented in Figure 1.

2. Additional subchronic toxicity studies from the sedaxane database on male rats

a) 90-Day Oral Study (MRID 47473375)

Two subchronic toxicity studies were conducted in rats with sedaxane and are included in the sedaxane database. One of these studies (Noakes, 2007) (MRID 47473375) was conducted in a different strain obtained from a different supplier than the carcinogenicity study, so it is only briefly mentioned here for completeness. In this study, Wistar rats (strain designation: HsdRccHan:WIST, from Harlan Lab, UK) were treated for 90 days with 0, 250, 1000 or 4000 ppm sedaxane in the diet. The dose level of 4000 ppm produced lower body weights, increased absolute and relative liver weights, and liver histopathology findings of centrilobular hypertrophy and increased pigmentation in males and females. The dose level of 1000 ppm produced minor increases in liver weight, but in the absence of any associated histopathology changes, this was not considered adverse. A smaller number of changes in clinical chemistry parameters were also observed at the 4000 ppm dose (including higher triglycerides, total protein), but there were no indicators of liver toxicity. The NOAEL in this study was 1000 ppm in males and females.

b) 90-Day Oral Study (MRID 47473376)

The second study, a 90-day study in rats (strain designation CrL:WI(Han), from Charles River Labs, UK), groups of 10 male and 10 female Han Wistar rats were fed diets containing 0, 300, 2000, or 4000 ppm sedaxane for 90 days (Shearer, 2009) (MRID 47473376). In male and female rats treated at 4000 ppm, body weight, body weight gain, and food consumption were significantly decreased. In female rats treated at 2000 ppm, body weight and body weight gain were also reduced. Sedaxane at 2000 ppm had no effect on male body weights, and 300 ppm was without effect on males or females.

Changes in clinical chemistry parameters that were considered treatment-related in male rats at 4000 ppm included slightly higher gamma glutamyl transferase (GGT), higher triglycerides, and higher total protein.

However, no clinical chemistry changes that would indicate liver toxicity were observed. The findings in this study (MRID 47473376) related to the proposed liver MOA are summarized in **Table 16**. Liver weights adjusted for body weight were statistically significantly higher than control values for 2000 and 4000 ppm males. Micropathology findings in the liver including centrilobular hypertrophy and hepatocyte pigment were only increased in the 4000 ppm males.

TABLE 16: Summary of data from a 90-day study (MRID 47473376) with sedaxane (liver-related parameters from male rats only)

	0 ppm	300 ppm	2000 ppm	4000 ppm
Liver Wt. (adjusted for BW) (g)	15.38	15.71	17.73**	20.94**
Liver Micropathology: (N)	(10)	(10)	(10)	(10)
Centrilobular hypertrophy	0	0	0	10***
Hepatocyte pigment	0	1	0	4

*, ** and ***: Statistically-significantly different from control with $p<0.05$, $p<0.01$ and $p<0.001$, respectively

CARC concluded that sedaxane treatment leads to an increase in liver weight (2000 and 4000 ppm) and hypertrophy (4000 ppm) in the rat (Associative Event for the mode of action hypothesis presented in Figure 1).

3. Additional subchronic toxicity studies from the sedaxane database in male mice

In CD-1 mice, subchronic toxicity studies were conducted as preliminary studies to facilitate dose level selection in the 18-month carcinogenicity study and are found within the sedaxane database. Very few of the measured parameters showed statistically significant or treatment-related changes; therefore, only a brief summary of the overall results is provided below.

a) 28-Day Oral Study (MRID 47473373)

In a 28-day study, CD-1 mice (strain designation: Crl:CD-1(ICR)) were treated with 0, 1000, 5000 and 7000 ppm sedaxane (Shearer and Robertson, 2008). There were no effects on body weight, liver weight or liver micropathology in male or female mice.

b) 90-Day Oral Study (MRID 47473377)

In a 90-day study, CD-1 mice (strain designation: Crl:CD-1(ICR)) were treated with 0, 500, 3500 and 7000 ppm sedaxane (Shearer and Foster, 2008). There were no effects on liver histopathology or clinical chemistry parameters that would indicate liver toxicity.

There were slightly higher, statistically significant liver weights in male mice were observed, but only after adjustment for body weight. The difference in adjusted liver weights in 7000 ppm males (+27%, $p<0.01$) was considered to be treatment-related, since the mean value was >25% higher than the control group mean, and histopathology changes were seen at 7000 ppm in the livers of mice treated for shorter durations. However, the smaller differences in adjusted liver weights at 500 ppm (+12%, $p<0.05$) and 3500 ppm (+12%, $p<0.05$) were not considered effects of treatment, considering the lack of any histopathology changes, no effects on absolute weights, and lack of a dose-response.

c) Hepatic Enzyme Activities after 28 and 90 Days of Dietary Administration to Male CD-1 Mice (MRID 49804823)

After the 28-day and a 90-day mouse studies were completed, further investigations with frozen liver samples were performed to examine the liver enzyme induction profiles (MRID 49804823). At termination, after necessary sections of the liver were taken for histology processing (formalin fixation), sections were collected for RNA isolation and enzyme activity determinations (MRID 47473373). Samples of liver for future use were taken in a similar manner from the 90-day mouse study (MRID 47473377) and stored at -70°C for future use.

The treatment of male mice with 1000 ppm and 7000 ppm sedaxane for 28 days had no statistically significant effect on hepatic whole homogenate protein content, cyanide insensitive palmitoyl-CoA oxidation activity, hepatic microsomal protein content, hepatic microsomal EROD activity, or hepatic microsomal lauric acid 12-hydroxylase activity. While treatment with 1000 ppm sedaxane had no statistically significant effect on microsomal total CYP content, treatment with 7000 ppm sedaxane significantly increased microsomal total CYP content to (+48%). The treatment of male mice with 1000 ppm and 7000 ppm sedaxane for 28 days significantly increased hepatic microsomal PROD activity to +133% and +1833% of control, respectively. The treatment of male mice with 1000 ppm and 7000 ppm sedaxane for 28 days had no statistically significant effect on hepatic microsomal testosterone 6β-hydroxylase activity. However, the hepatic microsomal testosterone 6β-hydroxylase activity for the 7000 ppm dose

group was +35% of the control value. The effects of treatment with sedaxane on male mice for 28 days on hepatic enzyme activities are presented in **Table 17**.

TABLE 17: Effect of treatment of male mice with sedaxane for 28 days on hepatic enzyme activities

	Sedaxane Treatment [a]		
	Control	**1000 ppm**	**7000 ppm**
Whole homogenate protein (mg/g liver)	196 ± 12.5	208 ± 2.3 (+6%)	202 ± 13.4 (+3%)
Palmitoyl-CoA oxidation (nmol/min/mg protein)	5.4 ± 0.44	4.4 ± 1.12 (-19%)	5.6 ± 1.03 (+4%)
Microsomal protein (mg/g liver)	22.6 ± 3.01	24.7 ± 3.85 (+9%)	23.5 ± 2.96 (+4%)
Cytochrome P450 content (nmol/mg protein)	0.23 ± 0.042	0.23 ± 0.052 (no change)	0.34 ± 0.047** (+48%)
7-Ethoxyresorufin O-deethylase (pmol/min/mg protein)	34 ± 8.4	28 ± 10.5 (-18%)	35 ± 4.2 (+3%)
7-Pentoxyresorufin O-dependentylase (pmol/min/mg protein)[b]	6 ± 1.9	14 ± 3.3** (+133%)	116 ± 26.4** (+1833%)
Testosterone 6β-hydroxylase (nmol/min/mg protein)	0.84 ± 0.238	0.80 ± 0.268 (-5%)	1.13 ± 0.323 (+35%)
Lauric acid 12-hydroxylase (nmol/min/mg protein)	0.47 ± 0.160	0.39 ± 0.081 (-17%)	0.55 ± 0.260 (+17%)

[a] Animals were fed control diet or diet containing sedaxane for 28 days.
Results are presented as mean ± SD for groups of 5 animals.
Values in parentheses are percentage of control levels.
[b] For statistical analysis data transformed by $\log_{10} y$.
** Values significantly different from control ($p<0.01$).

The treatment of male mice with 7000 ppm sedaxane for 90 days had no statistically significant effect on hepatic whole homogenate protein content, cyanide-insensitive palmitoyl-CoA oxidation activity, hepatic microsomal EROD activity, or hepatic microsomal lauric acid 12-hydroxylase activity. The treatment of male mice with 7000 ppm sedaxane for 90 days had no statistically significant effect on hepatic microsomal protein content, but significantly increased microsomal total CYP content (+40%). The treatment of male mice with 7000 ppm sedaxane for 90 days significantly increased hepatic microsomal PROD activity (+1300%). The treatment of male mice with 7000 ppm sedaxane for 90 days significantly increased hepatic microsomal testosterone 6β-hydroxylase activity (47%). Hepatic enzyme activities of male mice treated with sedaxane for 90 days is presented in **Table 18**.

TABLE 18: Effect of treatment of male mice with sedaxane for 90 days on hepatic enzyme activities

	Sedaxane Treatment [a]	
	Control	**7000 ppm**
Whole homogenate protein (mg/g liver)	213 ± 15.2	210 ± 12.1 (-1%)
Palmitoyl-CoA oxidation (nmol/min/mg protein)	6.1 ± 1.43	5.4 ± 0.95 (-11%)
Microsomal protein (mg/g liver)	24.0 ± 2.90	24.8 ± 2.26 (+3%)
Cytochrome P450 content	0.30 ± 0.033	0.42 ± 0.043**

(nmol/mg protein)		(+40%)
7-Ethoxyresorufin O-deethylase (pmol/min/mg protein)	36 ± 8.9	40 ± 6.5 (+11%)
7-Pentoxyresorufin O-depentylase (pmol/min/mg protein)	10 ± 3.7	140 ± 23.1** (1300%)
Testosterone 6β-hydroxylase (nmol/min/mg protein)	0.86 ± 0.164	1.26 ± 0.358** (+47%)
Lauric acid 12-hydroxylase (nmol/min/mg protein)	0.39 ± 0.075	0.38 ± 0.081 (-3%)

[a] Animals were fed control diet or diet containing sedaxane for 90 days.
Results are presented as mean ± SD for groups of 10 animals.
Values in parentheses are percentage of control levels.
** Values significantly different from control ($p<0.01$).

Based on the pattern of results observed, sedaxane did not appear to activate PPARα (peroxisome proliferator-activated receptor alpha). It also did not produce any increases in activity for palmitoyl-CoA oxidation or lauric acid 12-hydroxylase (an indicator of microsomal CYP4A induction), two enzymes that are known markers of PPARα activation (Klaunig *et al.*, 2003; Lake, 2009). Sedaxane also did not activate the aromatic hydrocarbon receptor (AhR). It did not produce any increases in EROD activity, which is a marker for CYP1A and CYP1B activities that are induced by AhR activation.

In contrast, sedaxane was an activator of CAR in mouse livers. PROD activity (a marker for CYP2B activity) was increased in a dose-responsive manner, with maximal activity that was 1933% and 1400% of the control value at 28 days and 90 days, respectively. It also produced a lesser induction of testosterone 6β-hydroxylase activity (a marker of CYP3A activity), but a treatment-related increase was observed only at 7000 ppm. This pattern of effects is consistent with the nuclear receptor reporter assays, which indicated that sedaxane was a CAR activator but not a PXR activator for mice (Elcombe *et al.*, 2014; Lake, 2009).

In terms of generalized markers of liver induction, sedaxane at 7000 ppm produced an increase in total CYP content, but it had no effects on total hepatic protein in either the microsomal fraction or the liver homogenate.

CARC concluded that sedaxane treatment leads to CAR activation (increase in CYP content, PROD activity, and testosterone 6β-hydroxylase activity) at 7000 ppm but not PXR activation in mice (Associative Event 1 for the mode of action hypothesis presented in Figure 1).

4. Non-neoplastic findings in a combined chronic toxicity and carcinogenicity study in Han Wistar male rats (Associative Events 2 & 3 and Key Event 5)

In addition to the tumor incidence data described in Section III A1, relevant toxicity data in the liver generated in the combined chronic toxicity and carcinogenicity study in rats (MRID 47473386) is presented in **Table 19** and **Table 20**. Body weight and body weight gain were decreased throughout the study in male rats at 3600 ppm. Male rats at 3600 ppm also had lower

food consumption during Weeks 1-7, but were comparable to controls thereafter (data not shown). After 52 weeks, dose-responsive increases in liver weights adjusted for body weight were observed at ≥1200 ppm, whereas the liver micropathology findings of centrilobular hypertrophy and hepatocyte pigment occurred only at 3600 ppm in male rats.

In the carcinogenicity phase of the study, terminal sacrifice animals at 104 weeks displayed increased liver weights, and the histopathology examination (including early decedents) revealed increased hepatocellular hypertrophy in males at ≥1200 ppm. In addition, the incidence of eosinophilic foci was statistically significantly higher at 3600 ppm, and this was considered a treatment-related effect.

TABLE 19: Summary of data from 52-week interim sacrifice in 2-year rat study (MRID 47473386) with sedaxane (non-neoplastic liver-related parameters from male rats)

		0 ppm	200 ppm	1200 ppm	3600 ppm
Weights:	**Week**				
Body weight gain (g) weeks 0 – 52	52	370.3	387.3	360.9	300.7** (-19%)
Liver wt. adjusted for body weight (g)	52	16.10	16.03	18.94**	22.63** (+41%)
Micropathology: (N)		(12)	(12)	(12)	(12)
Liver:					
Centrilobular hypertrophy	52	0	0	0	11***
Hepatocyte pigment	52	0	1	0	7**

Weights are mean and (percent of control).
*, ** and ***: Statistically-significantly different from control with $p<0.05$, $p<0.01$ and $p<0.001$, respectively. (Dunnett's test or Fisher's Exact Test).

TABLE 20: Summary of data from 104-week sacrifice + decedents in 2-year rat study (MRID 47473386) with sedaxane (non-neoplastic liver-related parameters from male rats)

		0 ppm	200 ppm	1200 ppm	3600 ppm
Weights:	**Week**				
Body weight gain (g) weeks 0 – 13	13	232.5	247.9*	228.0	187.3** (-19%)
Body weight gain (g) weeks 0 – 104	104	464.9	509.7*	447.9	355.7** (-23%)
Liver wt. adjusted for body weight (g)	104	18.10	18.06	20.21**	24.20** (+34%)
Micropathology: (N)		(52)	(52)	(52)	(52)
Liver:					
Centrilobular hypertrophy	104	0	0	8**	16***
Hepatocyte pigment	104	0	1	0	1
Eosinophilic cell focus	104	8	7	15	25***
Clear cell focus	104	33	39	37	19*
Angiectasis	104	0	1	6*	6*

Weights are mean and (percent of control).

*, ** and ***: Statistically-significantly different from control with p<0.05, p<0.01 and p<0.001, respectively. (Dunnett's test or Fisher's Exact Test).

CARC concluded that sedaxane treatment leads to hepatocellular hypertrophy (Associative Event 2), an increase in liver weight (Associative Event 3), and increased eosinophilic cell foci (Key Event 5) in the rat at 1200 and 3600 ppm for the mode of action hypothesis presented in Figure 1.

5. Non-neoplastic findings in a combined chronic toxicity and carcinogenicity study in male mice (Associative Event 3)

In addition to the tumor incidence data described in Section III B1, relevant toxicity data related to the liver that was generated in the 80-week carcinogenicity study in CD-1 male mice (MRID 47473388) is presented in **Table 21**. In the 80-week mouse study, lower body weights and body weight gains were observed for males (and females – data not shown) at 7000 ppm. The maximum difference from control for body weight was 7% in male mice at 7000 ppm. Food consumption was unaffected. Liver weight adjusted for body weight was statistically significantly increased in males at 7000 ppm (+16%). However, the report concluded that no histopathology changes in the liver were attributed to treatment with sedaxane.

In **Table 21**, all of the non-neoplastic histopathology changes in the liver that were tabulated in the original report are included for the male mice, summing all severities to give a total incidence.

TABLE 21: Summary of data from terminal sacrifice + decedents in 80-week mouse study with sedaxane (non-neoplastic liver-related parameters from male mice)

		0 ppm	200 ppm	1250 ppm	7000 ppm
Weights:	**Week**				
Body weight gain (g) weeks 0 – 13	13	14.0	13.0	13.7	12.3 (-13%)
Body weight gain (g) weeks 0 – 80	80	27.5	25.7	25.8	24.7 (-10%)
Liver wt. adjusted for body weight (g)	80	3.04	3.13	3.15	3.53* (+16%)
Micropathology:					
Liver (N):		(50)	(50)	(50)	(50)
Centrilobular hypertrophy	80	0	1	2	1
Periportal hypertrophy	80	0	0	0	0
Hepatocyte vacuolation	80	4	6	12*	8
Necrosis	80	2	4	3	3
Eosinophilic cell focus	80	3	1	3	3
Basophilic cell focus	80	2	1	3	6
Clear cell focus	80	4	4	7	3
Hemopoiesis, extramedullary	80	0	0	1	4*

Erythrophagocytosis, hepatocyte	80	0	1	0	0
Inflammatory cell infiltrate	80	1	1	0	1
Degeneration, hepatocyte	80	0	0	0	0

Weights are mean and (percent of control).
*, ** and ***: statistically-significantly different from control with $p<0.05$, $p<0.01$ and $p<0.001$, respectively. (Dunnett's test or Fisher's Exact Test).
Data from Perry (2010a) (MRID 47473388)

CARC concluded that sedaxane treatment leads to an increase in liver weight in <u>mice</u> at 7000 ppm (Associative Event 3 for the mode of action hypothesis presented in Figure 1).

IV. APPLICATION OF THE CANCER GUIDELINES MODE OF ACTION (MOA) FRAMEWORK FOR LIVER TUMORS

A. Postulated MOA and Key Events: *The following narrative was extracted from the registrant submitted MOA and human relevance framework document for rat and mouse liver tumors (MRID 49804809).*

Based on the available information, the registrant's representatives postulated that the MOA for sedaxane-induced rat and mouse liver tumors is **through activation of the constitutive androstane receptor (CAR)**.

<u>Key events for this MOA include the following:</u>
CAR/PXR activation
Altered expression of CAR-responsive genes
Altered expression of pro-proliferative genes
Transiently increased hepatocellular proliferation and decreased apoptosis
Clonal expansion and development of altered hepatic foci
Increase in liver tumor incidence compared to concurrent controls

<u>Associative events for this MOA include the following:</u>
Increased expression of genes encoding cytochrome P450s (CYPs), particularly Cyp2b and (to a lesser extent) Cyp3a isoforms
Increased hepatocellular hypertrophy
Increased liver weight

B. Dose-concordance of key events

Tables 22 summarizes the dose-concordance of the associative and causal key events for male rat liver tumors as proposed by the registrant. Overall, there is good dose concordance of the proposed key events with tumor outcome. With increasing doses, an increasing number of the key events are observed. Critically, it is only at the tumorigenic dose level (3600 ppm) that all of the causal and associative key events are observed.

TABLE 22. Summary of Dose-Concordance of Associative Events and (Causal) Key Events - Male Rat Liver Tumors

Dose of Sedaxane (ppm)[a]	CAR/PXR activation (Causal)	Induction of *Cyp* gene expression/ increased CYP activity[c] (Associative)	Increased hepatocellular hypertrophy (Associative)	Increased liver weight (Associative)	Induction of pro-proliferative and anti-apoptotic genes[d]	Transiently increased hepato-cellular proliferation (Causal)	Altered hepatic foci (Causal)	Higher Incidence of Combined Liver Tumors (EPA) Compared to Concurrent
200 (300) [a]	No data	No data	No	No	No data[d]	No data	No	No
500	Yes[b]	Yes	No	No	No data	No data		
1200	Yes[b]	Yes	Yes	Yes	No data	Yes	No	No
2000	Yes[b]	Yes	Yes	Yes	No data	No data	No data	No data
3600 (4000) [a]	Yes[b]	Yes	Yes	Yes	No data	Yes	Yes	Yes
5000	Yes[b]	Yes	Yes	Yes	No data	No data	No data	No data

a Values in parentheses are subchronic dose levels (that are similar to the chronic dose levels).
b Confirmed *in vitro* (CAR, PXR transactivation) and *in vivo* (dose levels inferred from observed increases in *cyp* gene expression and/or increased CYP activity).
c Increased CYP activity = primarily Cyp2b activity >> Cyp3a activity, based on PROD activity and Testosterone 16β-hydroxylase activity (Cyp2b markers) >> Testosterone 6β-hydroxylase activity (Cyp3a marker).
d Analysis of gene expression was not assessed for rats, but can be inferred from the large changes in 1) cell proliferation in rat hepatocytes, and 2) mouse RT-PCR and microarray data

Table 23 summarizes the dose-concordance of the key events for male mouse liver tumors as proposed by the registrant. In this comparison, dose levels of 7000 ppm and 14000 ppm are considered tumorigenic. Overall, the pattern of effects in the mouse liver were relatively weak in magnitude, compared with the rat, but the dose levels where the earlier key events were uniformly observed matched with the tumorigenic dose level of ≥7000 ppm.

TABLE 23. Summary of Dose-Concordance of Associative Events and (Causal) Key Events - Male Mouse Liver Tumors

Dose of Sedaxane (ppm)[a]	CAR activation (Causal)	Induction of *Cyp* gene expression/ increased CYP activity[c] (Associative)	Increased hepatocellular hypertrophy (Associative)	Increased liver weight (Associative)	Induction of pro-proliferative and anti-apoptotic genes [d] (Causal)	Transiently increased hepato-cellular proliferation (Causal)	Altered hepatic foci (Causal)	Statistically Higher Incidence of Combined Liver Tumors Compared to Concurrent Controls
200 (500) [a]	No data	No data	No	No	No data	No data	No	No
1250 (1000) [a]	Yes [b]	Yes	No	No	No [d]	No	No	No
3500 (5000) [e]	No data	No data	No	No	No data	No data	No data	No data
7000	Yes [b]	Yes	Yes/No [f]	Yes/No [f]	Yes [d]	Yes	No	Yes
14000	Yes [b]	Yes	Yes	Yes	Yes [d]	Yes	No data	No data

a Values in parentheses are subchronic dose levels (some of which are similar to the chronic dose levels of 200, 1250, or 7000 ppm).

b Confirmed *in vitro* (CAR transactivation) and *in vivo* (dose levels inferred from observed increases in *cyp* gene expression and/or increased CYP activity).

c Increased CYP activity = primarily Cyp2b activity >> Cyp3a activity, based on PROD activity (Cyp2b markers) >> Testosterone 6β-hydroxylase activity (Cyp3a marker).

d Gene expression changes in mouse liver that indicated a pro-proliferative, anti-apoptotic signal included increased Gadd45β expression, decreased Gadd45gamma expression, and further changes in the microarray KEGG pathway for cell cycle (Strepka, 2016).

e Dose levels of 3500 ppm and 5000 ppm were tested in a 90-day study and a 28-day study, respectively.

f At 7000 ppm, increases in liver weight and hepatocellular hypertrophy were observed only at certain time intervals and studies (See Time Concordance Table).

C. Toxicokinetic differences that correlate with difference in liver effects in mice vs. rats

The CAR reporter assays with sedaxane showed that sedaxane was an activator of rat, mouse and human CAR, and the relative fold-change at 30 μM in the different species indicated a greater response with mouse CAR (19-fold) than with rat CAR (6-fold) or human CAR (4-fold). Despite this apparent difference in the affinity for CAR receptor site between the species, sedaxane showed greater non-neoplastic liver responses at lower doses in male Wistar rats *in vivo* than in male CD-1 mice *in vivo*. **Tables 22** and **Table 23** provide an overview of the effects related to the liver at different doses (ppm in diet) for rats and mice, respectively. The magnitude of responses in mice to sedaxane for liver related parameters was generally weak, and this included the lack of apparent hepatocellular hypertrophy or liver weight increases at 28 days, lack of any eosinophilic foci in the 80-week study and a marginal increase in the incidence of a relatively common tumor type (hepatocellular adenoma + carcinoma) despite lifetime dosing at a limit dose (7000 ppm or approximately 1000 mg/kg/day). In addition, mice tolerated a dose as high as 14000 ppm in diet (equivalent to 2155 mg/kg/day) for 14 days with only a slight decrease in body weight gain and a small increase in liver weight, whereas the maximum dose that could be given to rats for repeated durations was 3600 – 4000 ppm (chronic or 90-day rat study).

Blood toxicokinetic data available from 14-day dosing studies in rats and mice suggests that differences in clearance of the parent molecule (isomers of sedaxane) between mice and rats may account for this apparent difference in sensitivity, as shown in **Table 24**, and suggested by the registrant. In the rat study, samples were taken every 4 hours starting at approximately 17 hours, which was approximately 8 hours after dietary feeding commenced. The rat T_{max} (time of the maximum blood concentration (C_{max})) appeared in the morning, which is consistent with overnight feeding, so that a comparison to the blood samples from the 14-day mouse study at termination provides a reasonable comparison of approximate steady-state C_{max} values. As shown in **Table 24**, the blood concentrations of the *trans*-isomer and the *cis*-isomer in male mice were less than the Limit of Quantitation (LOQ <10 ng/mL) in the majority of the mice, such that a reliable mean value could not be determined. This was the opposite of the pattern in male rats, where quantifiable C_{max} concentrations were observed consistently that were roughly proportionate to the administered dose. In a rat ^{14}C-sedaxane metabolism study (Green, 2009) the major circulating moieties in blood at a dose of 80 mg/kg were parent sedaxane (isomers) and a demethylated metabolite (*trans*- and *cis*-isomers). The HPLC analysis of mouse blood in the 14-day study detected only trace amounts of the desmethyl sedaxane isomers or of sedaxane isomers in a small proportion of the mice (MRID 49804822). These data suggest that sedaxane is rapidly metabolized to downstream metabolites and thus cleared from the blood, at a greater rate than in the rat, and this quantitative difference translates into less generalized toxicity (e.g. body weight decrease) and less effects on the liver (e.g. liver weight and hypertrophy increases) in mice than in rats, as suggested by the registrant.

TABLE 24: Comparison of blood concentrations of sedaxane isomers- short-term studies in male rats and male mice

Dose Level	Administered Dose (mg/kg/day)	Day	Mean C_{max}[a] *Trans* (SYN508210) (ng/mL)	Mean C_{max}[a] *Cis* (SYN508211) (ng/mL)
Male Rats:	**SYN524464 (50:50)**			
500 ppm	48	1	112	28
		14	60	32
2000 ppm	181	1	1220	101
		14	153	---
5000 ppm	445	1	1610	120
		14	613	32
Male Mice:	**SYN524464 (83.0:12.3)**			
7000 ppm	970	1	---	---
		14	<10	<10
10000 ppm	1389	1	---	---
		14	<10	<10
14000 ppm	2155	1	---	---
		14	<10	<10

--- = no data available

a Values are mean C_{max} from 28-day rat study (Peffer and Noakes, 2010) (MRID 47473372) and from a range finding 14-day mouse dietary study (MRID 49804822). In the rat study, samples were taken from eight hours after the administration of test diets and at four hour intervals thereafter (approximately 17.00, 21.00, 01.00, 05.00, 09.00 and 13.00 hours) on Day 1/2 and Day 14/15 of the study. Blood was centrifuged and the plasma was taken for analysis. In the mouse study, samples were taken by cardiac puncture under CO_2 anesthesia into EDTA tubes at the time of sacrifice. Whole blood samples were diluted with an equal volume of water and frozen prior to analysis. Rat plasma and mouse blood samples were analyzed by HPLC for determination of the *trans* isomer (SYN508210) and the *cis* isomer (SYN508211). T_{max} in rats was (in general) between 12 – 20 hr into the sampling regime, which equated to the morning hours (5.00 – 13.00 h). Thus, the sampling at a single sacrifice time for mice (morning) is considered a reasonable approximation of a C_{max} measurement.

D. Temporal-concordance of key events

The registrant concluded that the observed effects on parameters associated with the key events occur in a logical, time-dependent manner consistent with the proposed MOA. The temporal concordance for the rat tumor MOA is summarized in **Table 25**.

The observed steps occur in a logical sequence, with the key events preceding the time of occurrence of liver hepatocellular adenomas and carcinomas. In particular:

- Activation of CAR is inferred to occur after 1 day, based on a correlating increase in cell proliferation by BrdU labeling;
- This causal key event of hepatocellular proliferation was transiently affected, with significant increases at 1-3 days, but no measurable sustained increase above control at 7 days and longer;
- Induction of CYP activities (Cyp2b >> Cyp3a), increased hepatocellular hypertrophy, and increased liver weight occurred early (3-7 days), and remained consistently; affected over time. The CYP activities were assessed after 7 or 28 days of treatment, and they

would be expected to stay elevated after longer durations in concordance with the increased liver weights;
- A long-term consequence of these early key events included the postulated formation of altered hepatic foci (eosinophilic foci), which were only observed beyond one year; and
- A higher incidence of liver tumors vs. the concurrent control group required 1.5 – 2 years before it was observed.

While the activation of specific genes was not an endpoint measured in rat MOA studies, these data are available in the mouse MOA studies, and they can be inferred from the *in vitro* CAR/PXR transaction results, the CYP enzyme activity measurements and cell proliferation response in rat liver.

TABLE 25. Temporal Concordance of Associative Events and (Causal) Key Events in the Proposed MOA - Male Rat Liver Tumors

Time	CAR/PXR activation (Causal)	Induction of *cyp* gene expression/ increased CYP activity[b] (Associative)	Increased hepatocellular hypertrophy (Associative)	Increased liver weight (Associative)	Induction of pro-proliferative and anti-apoptotic genes [c]	Hepato-cellular proliferation (Causal)	Altered hepatic foci (Causal)	Higher Incidence of Combined Liver Tumors (EPA) Compared to Concurrent Controls (Outcome)
1 days	Yes[a]	No data	No	No	No data [c]	Yes	No	No
3 days	Yes[a]	No data	Yes	Yes	No data	Yes	No	No
7 days	Yes[a]	Yes	Yes	Yes	No data	No	No	No
28 days	Yes[a]	Yes	Yes	Yes	No data	No	No	No
90 days	No data	No data	Yes	Yes	No data	No [c]	No	No
1 year	No data	No data	Yes	Yes	No data	No data	No	No
1.5-2 years	No data	No data	Yes	Yes	No data	No data	Yes	Yes

a Confirmed *in vitro* (CAR, PXR transactivation) and *in vivo* (inferred from observed increases in cell proliferation, *cyp* gene expression and/or increased CYP activity).

b Increased CYP activity = primarily Cyp2b activity >> Cyp3a activity, based on PROD activity and Testosterone 16β-hydroxylase activity (Cyp2b markers) >> Testosterone 6β-hydroxylase activity (Cyp3a marker).

c Analysis of gene expression was not assessed for rats, but can be inferred from the large changes in 1) cell proliferation in rat hepatocytes, and 2) mouse RT- PCR and microarray data

The temporal concordance for the mouse liver tumor MOA is summarized in **Table 26**. The registrant concluded that the observed steps occur in a logical sequence, with the key events preceding the time of occurrence of liver hepatocellular adenomas and carcinomas. In particular:

- Activation of CAR is inferred to occur after 1 day, based on a correlating increase in gene expression (*Cyp2b10*, *Cyp2c65*, *Gadd45*β*);*
- Induction of CYP activities (Cyp2b >> Cyp3a), increased hepatocellular hypertrophy, and increased liver weight occurred early (1-7 days), and remained consistently affected over time;
- Differential expression of genes that promote a pro-proliferative and anti-apoptotic environment in the liver (e.g. increased *Gadd45β,* decreased *Gadd45*γ) also occurred early (detected at 1-7 days by RT-PCR; at 1-21 days by microarrays). The gradually diminished response with time for *Gadd45β* induction as well as *Cyp2b10* induction by RT-PCR is of interest. This pattern may be associated with a time-dependent greater metabolism of sedaxane by the induced CYP enzymes (discussed above);
- The diminished response for *Gadd45β* beyond 7 days is concordant with the transient effect on cell proliferation. The causal key event of hepatocellular proliferation was significantly increased after 7 days, but no measurable sustained increase was observed at 21 days;
- Despite the consistent increase in certain markers of CYP gene expression (*Cyp2c65*) or activity (PROD), the phenotypic endpoints of increased liver weight and liver hypertrophy were minimally affected or unaffected at 7000 ppm after longer time intervals; and
- A higher incidence of liver tumors vs. the concurrent control group required 80 weeks in male mice before it was observed.

The registrant concluded that the temporal relationships in mice are consistent with the proposed MOA and the known biology of CAR activators (Elcombe *et al.*, 2014). In addition, they point to a gradually diminished response in the livers of mice beyond 21 days, that is consistent with the relatively small increase in incidences vs. the concurrent control group of a fairly common tumor type in male CD-1 mice.

TABLE 26. Temporal Concordance of Associative Events and (Causal) Key Events at the Tumorigenic Dose Level and Higher - Male Mouse Liver Tumors

Time	CAR activation (Causal)	Induction of *cyp* gene expression/ increased CYP activity[b] (Associative)	Increased hepatocellular hypertrophy (Associative)	Increased liver weight (Associative)	Induction of pro-proliferative and anti-apoptotic genes [c] (Causal)	Hepato-cellular proliferation (Causal)	Altered hepatic foci (Causal)	Statistically Higher Incidence of Combined Liver Tumors Compared to Concurrent Controls (Outcome)
1 days	Yes [a]	Yes	Yes	No	Yes [c]	No	No	No
3 days	Yes [a]	Yes	Yes	Yes	Yes [c]	No	No	No
7 days	Yes [a]	Yes	Yes	Yes	Yes [c]	Yes	No	No
21 days	Yes [a]	Yes	Yes	Yes	Yes [c]	No	No	No
28 days	Yes [a]	Yes	No	No	No data	No data	No	No
90 days	Yes [a]	Yes	No	Yes	No data	No data	No	No
14 – 80 weeks	No data	No data	No	Yes	No data	No data	No	Yes

a Confirmed *in vitro* (CAR, PXR transactivation assays) and *in vivo* (inferred from observed increases in cell proliferation, *cyp* gene expression and/or increased CYP activity).

b Increased CYP activity = primarily Cyp2b activity >> Cyp3a activity, based on PROD activity (Cyp2b marker) >> Testosterone 6β-hydroxylase activity (Cyp3a marker).

c Gene expression changes in mouse liver that indicated a pro-proliferative, anti-apoptotic signal included increased *Gadd45β* expression, decreased *Gadd45γ* expression, and further changes in the microarray KEGG pathway for cell cycle (MRID 49804810).

E. Reproducibility and consistency

For both the rat and the mouse, where parameters were measured in multiple studies, the registrant concluded that there is a high degree of reproducibility between studies and consistency between key events. The first key event in the postulated MOA, activation of CAR (mice) or CAR and PXR (rat), was indicated in vitro in direct activation assays for CAR and for PXR, and it was evident in multiple in vivo studies based on increases in Cyp2b gene expression and Cyp2b enzymatic activity (both PROD and testosterone 16β-hydroxylase activities). The liver weight increases and associated hepatocellular hypertrophy were consistently observed in all of the rat in vivo studies; in mice, these phenotypic changes were much less affected by 7000 ppm sedaxane at later times. However, use of a dose (14000 ppm) higher than the tumorigenic dose produced stronger responses in the 21-day mechanistic study, giving greater confidence that the sequence of key events is well understood. The transient increase in hepatocellular proliferation was observed in both rats and in mice, and corresponding changes in gene expression in mice that indicate a pro-proliferative environment are further support for this key event. Finally, the matching key events and similar overall MOA pathways in Wistar rats and CD-1 mice provides a strong measure of reproducibility across similar rodent species.

The only minor inconsistency in the database is the lack of an increase in eosinophilic foci in the 80-week mouse study. This late key event was clearly observed at the tumorigenic 3600 ppm dose in male rats. However, it is plausible that an increase in altered foci in the mouse liver may have preceded the development of tumors, but it could not be observed because no interim sacrifice is made in a Guideline mouse carcinogenicity study. In addition, this minor difference between the species is consistent with the overall pattern of much milder non-neoplastic effects on the liver occurring in mice than in rats, likely due to differences in toxicokinetics.

F. Biological plausibility

The liver is the most common target tissue affected in carcinogenicity studies in rodents (Gold et al., 2001). This may be due to the fact that the liver is the major site of metabolic processing of xenobiotics, as well as being the first organ exposed following absorption from the gastrointestinal tract (if administered orally, as in the case of the carcinogenicity studies with sedaxane). The induction of liver tumors in male mice subsequent to the activation of CAR is a comprehensively studied and characterized MOA for a number of compounds, including the archetypal CAR activator phenobarbital (Elcombe et al., 2014; Meek et al., 2003; Whysner et al., 1996) and the potent mouse CAR activator TCPOBOP (Huang et al., 2005). In addition, the stated MOA and the clear dependence on CAR activation as a causal key event is consistent with the data for other SDHI fungicides that produce rodent liver tumors via this MOA, including fluopyram (ECHA, 2012; U.S. EPA, 2014a), penflufen (U.S. Environmental Protection Agency, 2011c), and fluxapyroxad (U.S. Environmental Protection Agency, 2011a).

G. Alternative mode of action hypotheses

In addition to CAR/PXR activation, a number of alternative MOAs for induction of liver tumors in rodents and/or humans have been demonstrated (Cohen, 2010). These alternative MOAs proposed by the registrant, and the reasons why they can be excluded for sedaxane, are described below (**Table 27**).

TABLE 27: Alternative Modes of Action for Induction of Liver Tumors in Rodents and Reason(s) for their Exclusion for Sedaxane

Alternative MOA	Reason for exclusion
Genotoxicity	Sedaxane has been tested in a wide variety of *in vitro* and *in vivo* assays for genotoxicity. There is no evidence that sedaxane is genotoxic (**Table 28**).
Peroxisome proliferator	Treatment with sedaxane did not increase male mouse hepatic peroxisomal fatty acid β-oxidation or lauric acid 12-hydroxylation activity (a marker of Cyp4a activity) (MRID 49804823). Also, in a rat 28-day study, electron microscopy of the livers showed no evidence of peroxisome formation (Peffer and Noakes, 2010) (MRID 47473372).
Enzyme induction (aryl hydrocarbon receptor [AhR]-mediated)	Treatment with sedaxane did not result in increased EROD activities in rats or in mice (MRID 49804823; MRID 47473372). In addition, no strong induction of Cyp1a isoform expression of the magnitude seen with AhR activators was observed in mouse liver microarrays (MRID 48804810).
Estrogenic stimulation	In the large mammalian toxicological database available for sedaxane, including the studies summarized in this document, as well as studies of the effects of sedaxane on reproduction and development (Peffer and Parr-Dobrzanski, 2010) (MRID 47473511), there is no evidence for estrogenic stimulation. In addition, sedaxane showed no estrogenic activity in a rat uterotrophic assay (MRID 49804803).
Statins	Sedaxane was not designed to inhibit HMG-CoA reductase. Also, the toxicology study database shows that cholesterol levels are mildly increased in rats by sedaxane treatment (not decreased) and there were no effects of sedaxane on cholesterol levels in 28 and 90-day studies in mice (Peffer and Parr-Dobrzanski, 2010) (MRID 47473511).
Cytotoxicity	Following administration to rats and mice, sedaxane did not produce elevations in markers of hepatocyte damage nor was there any evidence of cytotoxicity or regenerative proliferation (the proliferation noted following treatment with sedaxane was transient and not sustained).
Infection	Following administration to rats and mice, sedaxane did not produce any signs of hepatic infection, cytotoxicity or regenerative proliferation. The proliferation noted following treatment with sedaxane was transient and not sustained.
Iron/copper overload	Following administration to rats and mice, sedaxane did not produce elevations in markers of hepatocyte damage, or specific staining of tissues reflective of iron deposition, nor was there any evidence of cytotoxicity or regenerative proliferation.
Increased apoptosis	There was no consistent evidence that administration of sedaxane increased hepatic apoptosis in rats or in mice.

The CARC previously concluded that there is no mutagenic concern for sedaxane (TXR #0055706). Since that time an unscheduled DNA synthesis study (Hall, 2011) (MRID 50102201) was submitted and determined to be negative (**Table 28**).

TABLE 28: Summary of genotoxicity studies with sedaxane

Study	Dose Levels	Result
***In vitro* studies**		
Bacterial reverse mutation (Sokolowski, 2009) (MRID 47473381)	3 – 5000 µg/plate	Negative
In vitro cytogenetics (Bohnenberger, 2009) (MRID 47473382)	23.1 – 216.8 µg/mL	Negative
Mammalian cell gene mutation (mouse lymphoma) (Wollny, 2009) (MRID 47473383)	6.9 – 110 µg/mL	Negative
***In vivo* studies**		
Mouse bone marrow micronucleus (Reichenbach, 2010) (MRID 47473384)	2000 mg/kg	Negative
Unscheduled DNA synthesis – rat liver (Durward, 2009) (MRID 47473385)	2000 mg/kg	Negative
Unscheduled DNA synthesis – rat liver (Hall, 2011) (MRID 50102201)	1000 – 2000 mg/kg	Negative [New study, part of this submission]

G. Uncertainties, inconsistencies and data gaps

The registrant concluded that the available data strongly support the proposed MOA for induction of rat and mouse liver tumors by sedaxane (**Figure 1**) and exclude the alternative MOAs described in **Table 27**. No critical uncertainties or inconsistencies have been identified.

V. ARE THE KEY EVENTS IN THE ANIMAL MODE OF ACTION FOR LIVER TUMORS PLAUSIBLE IN HUMANS?

Following establishment of a plausible MOA for the induction of liver tumors in mice, the next step is to assess the relevance to humans by assessing the qualitative and quantitative differences between the mouse and human for each of the key events. As described in a recent extension of the IPCS Mode of action Framework (Boobis *et al.*, 2006), the questions to be asked are:

• Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

• Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?

A. Qualitative differences in key events

Sedaxane was shown to be a direct activator of mouse, rat, and human CAR, and of rat and human PXR *in vitro*. Therefore, it can be concluded that the human is not qualitatively different to the mouse and rat with respect to the initial causal key event of CAR/PXR activation following sedaxane treatment, although quantitative differences were observed. The magnitude of the response in the CAR and PXR transactivation assays indicated that CAR (6.3-fold) is more responsive than PXR (3.1-fold) to sedaxane in rats, and that in mice, only CAR activation was observed (19-fold). The *in vivo* liver responses in mice and rats are concordant with CAR activation being the primary initiator of liver changes in rats and mice, with markers of Cyp2b activity being much greater than Cyp3a markers of activity in sedaxane-treated mice and rats. On this basis, the following discussion will focus primarily on the human response to CAR activation. **Figure 7** presents a summary of interspecies differences in response to sedaxane.

A number of studies have shown that CAR can be activated by compounds such as phenobarbital in the mouse, rat, Syrian hamster, non-human primate, and human, resulting in altered gene expression, hypertrophy, and CYP2B enzyme induction (Diwan *et al.*, 1986; Elcombe *et al.*, 2014; Huang *et al.*, 2005; Olsen *et al.*, 1989; Weaver *et al.*, 1994; Yamamoto *et al.*, 2004). In contrast, while phenobarbital enhances cell proliferation and decreases apoptosis in the mouse and rat, other species appear to be refractory to the proliferative and anti-apoptotic responses. For example, phenobarbital has been reported not to stimulate DNA synthesis and not to inhibit apoptosis in cultured Syrian hamster and guinea pig hepatocytes (James and Roberts, 1996). In keeping with the lack of effect of phenobarbital on cell proliferation (Parzefall *et al.*, 1991) in the Syrian hamster, chronic phenobarbital treatment does not produce liver tumors in this species when given in the drinking water at 500 ppm (Diwan *et al.*, 1986).

Although phenobarbital can increase liver size in both rodents and humans (Aiges *et al.*, 1980), significant species differences in the mitogenic and anti-apoptotic properties of phenobarbital and related compounds have been demonstrated. In contrast to effects in cultured rodent hepatocytes, phenobarbital does not induce replicative DNA synthesis and does not inhibit apoptosis in human hepatocytes (Hasmall and Roberts, 1999; Hirose *et al.*, 2009). While phenobarbital can act as a non-genotoxic carcinogen and tumor promoter in the rat and mouse, it does not appear to produce liver tumors in humans. A number of epidemiological studies have demonstrated that in human subjects receiving phenobarbital for many years at doses producing plasma concentrations similar to those that are carcinogenic in rodents, there is no evidence of increased liver tumor risk (Friedman *et al.*, 2009; IARC, 2001; Olsen *et al.*, 1989; Olsen *et al.*, 1995; Whysner *et al.*, 1996).

To explore the species differences in response to sedaxane, an *in vitro* investigative study using primary hepatocytes isolated from male Wistar rats was conducted to assess the effects of sedaxane on PROD and BROD activities and hepatocellular proliferation (Vardy, 2016a) (MRID

49804812), and a similar experiment was conducted with isolated male human hepatocytes (Vardy, 2016b) (MRID 49804811).

In these experiments, hepatocytes were exposed for 96 hours to 6 concentrations of sedaxane, up to a maximum of 100 µM. A value of 100 µM was estimated to be the highest concentration that could be tested based on cytotoxicity that had been observed in prior *in vitro* studies with sedaxane. Testing up to the highest tolerable concentration allows for a robust assessment of the intrinsic potential of sedaxane to induce CYP2B/3A activity and hepatocellular proliferation. These experiments also included appropriate controls: sodium phenobarbital as a known inducer of PROD/BROD activities in both species and an inducer of cell proliferation in rat, and epidermal growth factor (EGF) as a known inducer of cell proliferation in both species.

Table 29 provides a summary of the data from the two studies and shows that treatment with sedaxane caused induction of CYP2B/3A activity (observed as an increase in PROD and BROD activity) and a proliferative response in the rat hepatocytes. In contrast, sedaxane did not produce a cell proliferation response in the human hepatocytes. It did cause an increase in BROD activity. Both control compounds gave the expected responses for both species indicating the test systems were responding as expected.

TABLE 29: Results of Primary Hepatocyte Mode of Action Studies with Sedaxane

Species	CYP induction[a]	Cell proliferation	Reference
Rat Hepatocytes	↑ PROD activity and ↑ BROD activity	↑ S-phase labelling index	(Vardy 2016a) (MRID 49804812)
Human Hepatocytes	↑ BROD activity No effect on PROD activity	None	(Vardy 2016b) (MRID 49804811)

[a] Abbreviations:
pentoxyresorufin-O-dependentylation (PROD) is a mainly a marker for CYP2B activity.
benzyloxyresorufin-O-debenzylation (BROD) is mainly a marker for CYP2B/3A activity

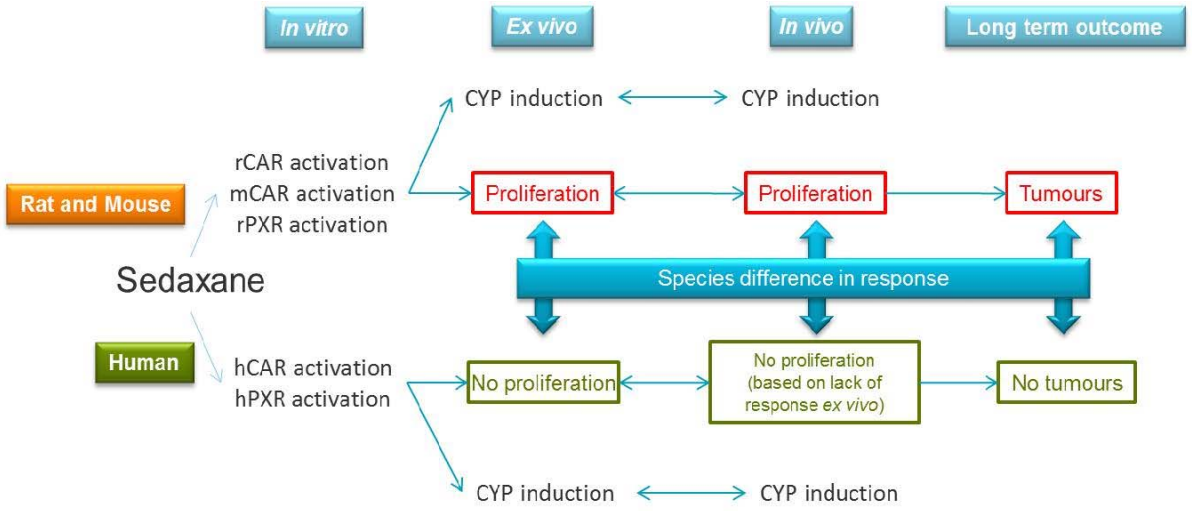
Therefore, based on experimental data, human hepatocytes have been shown to be non-responsive to sedaxane regarding the causal key event of cell proliferation. This pattern of effects matches the known species differences that have been demonstrated for phenobarbital and other CAR activators, and the weight of evidence indicates that it represents a qualitative difference in the established MOA for sedaxane between rodents (rats and mice) and humans (Elcombe *et al.*, 2014) (**Figure 7**). Therefore, it can be concluded that the tumorigenic MOA established for sedaxane in male rats and male mice is not operative in humans based on qualitative differences between rodents and humans in their response to sedaxane.

Therefore, the registrant concluded, the answer to the question "Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?", is clearly "Yes" for sedaxane. However, the

Agency concluded that the human CAR receptor is activated by sedaxane as seen *in vitro* studies (Figure 2), and therefore, can be relevant to humans qualitatively. In addition, there is limited data to determine if *in vivo* human hepatocyte models would continue to express receptors in culture. Furthermore, the statement does not take into consideration diversity that occurs across human population subgroups.

Considering that a qualitative difference has been established, the question of quantitative differences in key events between experimental animals and humans does not need to be reviewed in this assessment. However, demonstration of the MOA *via* CAR/PXR activation in rats and mice with sedaxane has also established that all of the causal key events are only operative at the tumorigenic dose levels in the respective species, and that clear thresholds exist for these effects in rats and mice. Therefore, human health risk assessments can be conducted using a Margin of Exposure approach for all effects, including neoplastic or non-neoplastic changes.

Figure 7: Summary of Interspecies Differences in Response to Sedaxane



VI. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE FOR THE CAR MOA FOR LIVER TUMORS

On February 22, 2017, CARC reconvened to evaluate the submissions of new studies containing MOA data on sedaxane. From these deliberations, the CARC drew the following conclusions for the sedaxane-induced rat and mouse liver tumor MOA:

- **There is evidence that sedaxane causes direct activation of CAR (mouse, rat, and human) and PXR (rat and human) nuclear receptors as evidenced by *in vitro* gene expression. There is *in vivo* data supporting an increase in microsomal protein content (rat), UGT activity (rat), total CYP content (rat and mouse), PROD activity (rat and mouse), testosterone 16β-hydroxylase activity (rat), and testosterone 6β-hydroxylase activity (rat and mouse). RT-PCR data presented an increase in Gadd45β mRNA levels (mouse). In addition, microarray data showed an increase in expression of xenobiotic metabolizing enzymes and other genes associated with CAR/PXR activation (mouse).**

- **The data support the associative events of increased liver weight and hepatocellular hypertrophy.**

- **There is evidence of sedaxane induced hepatocellular proliferation in rats and mice. Although there is a lack of increased cell proliferation in the mouse by BrdU labelling index, there is an increase in Ki67 labelling index by day 8 in this species. In addition, there is a robust proliferative burst in the rat BrdU labelling experiment and strong evidence for CAR induction as noted above.**

- **There is evidence of increased altered eosinophilic foci in the rat. There is a lack of increase in eosinophilic foci in the 80-week mouse study; however, given the modest tumorigenic response in the male mouse, along with the known concordance in the CAR MOA between the mouse and rat, the concern is lessened.**

- **There is good concordance between the dose causing tumors (3600 ppm male rats; 7000 ppm male mice) and the dose response and temporal associations for the key and associative events.**

- **Alternative MOAs (*i.e.,* genotoxicity, cytotoxicity, peroxisome proliferation, estrogenic stimulation, statins, infections, iron/copper overload, increased apoptosis, and mitogenesis induced by other nuclear receptors such as AhR or PPAR α) have been adequately ruled out.**

  Conclusion: There is plausible evidence that the mode of action (MOA) for the rat and mouse liver tumors induced by sedaxane is mitogenesis through activation of CAR/PXR nuclear receptors.

VII. EVALUATION OF THYROID TUMORS AND MECHANASTIC STUDIES

As stated above, CARC previously considered the thyroid tumors to be weak evidence of a treatment-related effect only at the high dose (3600 ppm) in male rats, based on the following information:

A. Thyroid Tumors in Male Rats:

The following text in Section VII. A-C was extracted directly from the first CARC meeting (March 16, 2011) report (TXR #0055706).

In a combined chronic toxicity/carcinogenicity study, 52 Crl:WI(Han)(Han Wistar) rats/sex/dose were exposed to sedaxane (95.3% a.i.) for up to 2 years in the diet at concentrations of 0, 200, 1200, or 3600 ppm (equivalent to 0/0, 11/14, 67/86, and 218/261 mg/kg bw/day in males/females, respectively) (MRID 47473386). An additional 12 rats/sex/dose were treated similarly for up to 1 year and then sacrificed.

Data from the tumor analyses are presented in **Table 30**. No thyroid tumors were seen in female rats. For male rats, statistically significant trends only were seen for follicular cell adenomas and combined adenomas and/or carcinomas, both at $p<0.05$. There were no significant pair-wise comparisons of the dosed groups with the controls for adenomas, carcinomas or combined tumors. The combined tumor increase was driven mainly by the adenomas (i.e., no malignant component).

Table 30. Sedaxane – Crl:WI(Han)(Han Wistar) Male Rat Thyroid Follicular Cell Tumor Rates[+]

	Dose (ppm)			
	0	**200**	**1200**	**3600**
Adenomas (%) p =	3[a]/52 (6%) 0.0299*	3/50 (6%) 0.6421	4/52 (8%) 0.5000	8/52 (15%) 0.1004
Carcinomas (%) p =	0/52 (0%) 0.2056	0/50 (0%) 1.0000	2[b]/52 (4%) 0.2476	1/52 (2%) 0.5000
Combined (%) p =	3/52 (6%) 0.0182*	3/50 (6%) 0.6421	6/52 (12%) 0.2439	9/52 (17%) 0.0611

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.
[a]First adenoma observed at week 87, dose 0 ppm.
[b]First carcinoma observed at week 87, dose 1200 ppm.

Note: Significance of trend denoted at control (the p values on the control groups are the trends).
Significance of pair-wise comparison with control (dose 0) denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

B. Other Related Toxic Effects in Male Rats

In addition to the thyroid tumors discussed above, increased incidences of thyroid follicular cell hypertrophy and epithelial desquamation were seen in both sexes dosed at 1200 ppm and above.

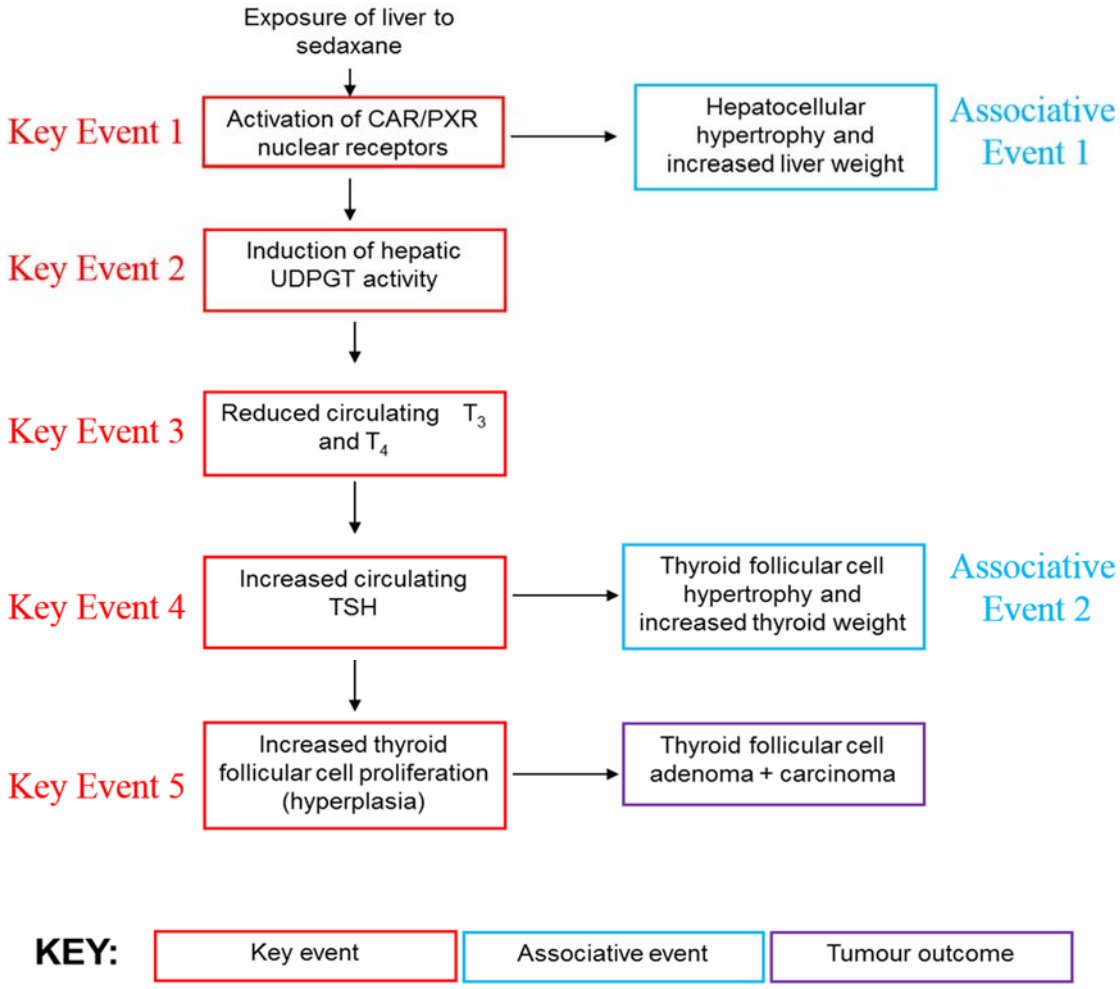
C. CARC Conclusions on Male Rats:

From these data, the CARC consider the thyroid tumors to be weak evidence of a treatment-related effect only at the high dose (3600 ppm) in male rats.

D. Mechanistic studies

A mode of action has been postulated by the Registrant (MRID 49804818) for sedaxane-induced rat thyroid tumors. The key events in the proposed MOA include: activation of the CAR/PXR nuclear receptors and induction of hepatic UDP- glucuronosyltransferase (UDPGT) [referred to as UGT in the remainder of the document], resulting in increased conjugation and excretion of triiodothyronine (T_3) and thyroxine (T_4), a decrease in serum T_3 and T_4 levels, and a compensating increase in thyroid stimulating hormone (TSH) levels secreted *via* the hypothalamus-pituitary-thyroid (HPT) axis. Under the chronic proliferative stimulus of TSH, thyroid follicular cells undergo hypertrophy and hyperplasia, and eventually progress to form follicular cell adenomas and/or carcinomas (**Figure 8**).

Figure 8: Mode of Action Hypothesis for the Induction of Thyroid Tumors in Rats



1. **Mechanistic studies submitted to support proposed MOA**

a) CAR and PXR activation assays (MRID 49804825 and MRID 49804824) (Key Event 1)

See Section III C1a for data related to CAR and PXR activation. In addition, consistent with its activity as a CAR/PXR activator, sedaxane caused dose-dependent increases in PROD activity (see Section III C1b). PROD was only measured on Day 8 samples, but would be assumed to have increased at each time point when liver weights were increased; PROD is a marker of Cyp2b enzyme activity, which is induced by CAR and PXR nuclear receptor activation.

CARC concluded that sedaxane activates mouse, rat, and human CAR in addition to rat and human PXR (Key Event 1 for the mode of action hypothesis presented in Figure 8).

b) Induction of hepatic UGT activity in sedaxane-treated male rats (Key Event 2)

28 Day Oral (Dietary) Mechanistic Study to Evaluate Effects on the Liver and Thyroid on the Male Rat (MRID 4984819)

Please see Section III C1b for a review of study conditions. Treatment with 1200 and 3600 ppm sedaxane for 1, 3, 7, 14, and 28 days significantly increased UGT activity towards thyroxine as substrate expressed as specific activity, per gram of liver, per total liver and per relative liver weight. The hepatic enzyme induction effects of sedaxane were reversible, as the statistically significant increases in UGT activity towards thyroxine as substrate that were observed after 28 days of sedaxane treatment were no longer observed following the recovery period. Small but statistically significant decreases in UGT activity were observed when enzyme activity was expressed per gram of liver, per total liver and per relative liver weight after the recovery period, but considering the direction of change versus control, these small decreases in hepatic UGT activity are not considered to be of any toxicological significance. Treatment with 1200 ppm phenobarbital as a positive control for 3, 7, 14 and 28 days resulted in significant increases in UGT activity towards thyroxine as substrate expressed as specific activity, per gram of liver, per total liver and per relative liver weight (**Table 4**).

CARC concluded that sedaxane treatment at 1200 and 3600 ppm leads to an increase in hepatic UGT activity in the rat (Key Event 2 for the mode of action hypothesis presented in Figure 8).

c) Hepatocellular hypertrophy and increased liver weight in sedaxane-treated rats

28 Day Oral (Dietary) Mechanistic Study to Evaluate Effects on the Liver and Thyroid on the Male Rat (MRID 4984819) (Associative Event 1)

Please see Section III C1e for data supporting an increase in liver weight and histopathology in sedaxane-treated male rats.

CARC concluded that sedaxane treatment leads to an increase in liver weight and hepatocellular hypertrophy at 1200 and 3600 ppm in the rat (Associative Event 1 for the mode of action hypothesis presented in Figure 8).

d) Reduced circulating T_3 and T_4 and increased circulating TSH in sedaxane-treated rats (Key Events 3 & 4)

28 Day Oral (Dietary) Mechanistic Study to Evaluate Effects on the Liver and Thyroid on the Male Rat (MRID 4984819)

Study conditions have been described earlier. The data generated in this study with the positive control compound (sodium phenobarbital) support a mode of action in the rat secondary to increased hepatic clearance of thyroid hormones. Decreases in total T_3 (**Table 33**) and total T_4

(**Table 34**) were observed with sodium phenobarbital treatment at most time-points, beginning on day 2, while elevations of TSH (**Table 35** and **Table 36**) were observed at later times (i.e. days 8, 15 and 29), consistent with the temporal nature of thyroid hormone and TSH homeostasis. Free T_3 and T_4 are also presented in **Table 31** and **Table 32**, respectively.

The changes in the sedaxane-treated groups suggested a similar thyroid mode of action but the effects on TSH and T_4 were less clear in these groups. Total T_3 showed a statistically significant decrease in one or both sedaxane treatment groups on days 2, 4, 8 and 15 (**Table 33**). Total T_4 was statistically significantly decreased by treatment with sedaxane only at day 2 but some low individual values were also apparent at day 4 (1200 and 3600 ppm groups) (**Table 34**).

TSH concentrations in sedaxane-treated groups were in general highly variable between individual animals, and the mean TSH values, though numerically higher than controls on days 15 and 29, were not statistically significant (Table 38). Therefore, based on these observations, a marginal increase in TSH could possibly be present after 14 - 28 days of sedaxane treatment, the same time interval when TSH values were highest in the positive control group. However, definitive increases in TSH levels for sedaxane-treated groups were not discernible for the time points that were assessed in this study.

TABLE 31. Free T_3 Group Mean Concentrations [a]

Dose rate ppm	Free T_3 (pmol/L)					
	Day 2	**Day 4**	**Day 8**	**Day 15**	**Day 29**	**Day 89**
0	4.455 ± 0.997	5.265 ± 0.689	4.707 ± 0.284	5.235 ± 0.737	4.899 ± 0.470	6.413 ± 0.485
1200 ppm sedaxane	6.447 ± 0.651* (+45%)	10.277 ± 0.610* (+95%)	5.433 ± 0.356 (+15%)	8.718 ± 0.938* (+67%)	8.665 ± 1.309* (+77%)	NA
3600 ppm sedaxane	5.709 ± 0.415* (+28%)	9.617 ± 1.116* (+83%)	6.845 ± 1.951* (+45%)	10.151 ± 1.197* (+94%)	8.925 ± 1.222* (+83%)	8.246 ± 0.862* (+26%)
1200 ppm positive control (sodium phenobarbital)	5.365 ± 0.413* (+20%)	8.887 ± 0.589* (+69%)	8.963 ± 0.640* (+90%)	9.212 ± 1.208* (+76%)	7.926 ± 0.716* (+62%)	NA

[a] Data obtained from pages 644 & 650 in the study report (MRID 4980419).
* Statistically different ($p < 0.01$) from the control (Dunnett 2-sided test).
NA= data not available
Percent changes are presented in parentheses and were calculated by the reviewer.

TABLE 32. Free T_4 Group Mean Concentrations [a]

Dose rate ppm	Free T_4 (ng/dL)					
	Day 2	Day 4	Day 8	Day 15	Day 29	Day 89
0	2.161 ± 0.325	2.138 ± 0.209	1.801 ± 0.342	2.140 ± 0.142	1.901 ± 0.288	1.749 ± 0.183
1200 ppm sedaxane	2.649 ± 0.220* (+23%)	2.405 ± 0.335** (+12%)	2.174 ± 0.145* (+21%)	2.167 ± 0.222 (+1.3%)	2.371 ± 0.348* (+25%)	NA
3600 ppm sedaxane	2.179 ± 0.197 (+0.8%)	2.720 ± 0.264* (+27%)	2.571 ± 0.341* (+43%)	2.807 ± 0.288* (+31%)	3.167 ± 0.333* (+67%)	2.097 ± 0.222* (+20%)
1200 ppm positive control (sodium phenobarbital)	1.890 ± 0.285** (-12.5%)	2.001 ± 0.127** (-6%)	2.138 ± 0.118* (+19%)	2.011 ± 0.186** (-6%)	1.891 ± 0.352(-0.5%)	NA

[a] Data obtained from pages 645 & 651 in the study report (MRID 4980419).
* Statistically different ($p < 0.01$) from the control (Dunnett 2-sided test).
** Statistically different ($p < 0.05$) from the control (Dunnett 2-sided test).
NA= data not available
Percent changes are presented in parentheses and were calculated by the reviewer.

TABLE 33. Total T_3 Group Mean Concentrations [a]

Dose rate ppm	Total T_3 (ng/mL)					
	Day 2	Day 4	Day 8	Day 15	Day 29	Day 89
0	1.287 ± 0.159	1.125 ± 0.149	1.235 ± 0.294	1.118 ± 0.196	0.883 ± 0.163	1.149 ± 0.268
1200 ppm sedaxane	1.132 ± 0.217 (-12%)	0.908 ± 0.147* (-19%)	0.673 ± 0.089* (-46%)	0.797 ± 0.186* (-29%)	1.147 ± 0.329** (+30%)	NA
3600 ppm sedaxane	0.945 ± 0.198* (-27%)	0.944 ± 0.199* (-16%)	0.923 ± 0.278* (-25%)	0.582 ± 0.138* (-48%)	1.285 ± 0.337* (+46%)	0.943 ± 0.234** (-18%)
1200 ppm positive control (sodium phenobarbital)	0.879 ± 0.249* (-32%)	0.725 ± 0.133* (-36%)	0.849 ± 0.132* (-31%)	0.514 ± 0.139* (-54%)	1.205 ± 0.246* (+36%)	NA

[a] Data obtained from pages 646 & 652 in the study report (MRID 4980419).
* Statistically different ($p < 0.01$) from the control (Dunnett 2-sided test).
** Statistically different ($p < 0.05$) from the control (Dunnett 2-sided test).
NA= data not available
Percent changes are presented in parentheses and were calculated by the reviewer.

TABLE 34. Total T_4 Group Mean Concentrations [a]

Dose rate ppm	Total T_4 (ug/dL)					
	Day 2	Day 4	Day 8	Day 15	Day 29	Day 89
0	3.865 ± 0.990	4.013 ± 0.529	3.191 ± 1.006	4.240 ± 0.424	3.355 ± 0.548	3.708 ± 0.854
1200 ppm sedaxane	3.666 ± 0.711 (-5%)	3.428 ± 0.603 (-15%)	3.984 ± 0.751** (+25%)	3.823 ± 1.033 (-10%)	3.935 ± 0.871 (+17%)	NA
3600 ppm sedaxane	2.725 ± 0.555* (-29%)	3.526 ± 0.910 (-12%)	3.975 ± 0.887** (+25%)	5.248 ± 0.906* (+24%)	3.515 ± 0.706 (+5%)	3.546 ± 0.726 (-4%)
1200 ppm positive control (sodium phenobarbital)	3.137 ± 0.823** (-19%)	2.539 ± 0.604* (-37%)	2.385 ± 0.630** (-25%)	4.059 ± 0.602 (-4%)	2.820 ± 0.634** (-16%)	NA

[a] Data obtained from pages 647 & 653 in the study report (MRID 4980419).
* Statistically different ($p < 0.01$) from the control (Dunnett 2-sided test).
** Statistically different ($p < 0.05$) from the control (Dunnett 2-sided test).
NA= data not available
Percent changes are presented in parentheses and were calculated by the reviewer.

TABLE 35. ELISA TSH Group Mean Concentrations [a]

Dose rate ppm	TSH Mean (ng/mL)					
	Day 2	Day 4	Day 8	Day 15	Day 29	Day 89
0	0.666 ± 0.315	1.704 ± 0.789	0.755 ± 0.502	1.486 ± 0.194	1.283 ± 0.396	0.545 ± 0.259
1200 ppm sedaxane	0.886 ± 0.397 (+33%)	0.973 ± 0.336** (-43%)	1.158 ± 0.622** (+53%)	1.976 ± 2.235 (+33%)	0.997 ± 0.841 (-22%)	NA
3600 ppm sedaxane	0.565 ± 0.272 (-15%)	0.824 ± 0.400* (-52%)	0.744 ± 0.335 (-1.5%)	0.695 ± 0.441** (-53%)	1.173 ± 0.557 (-9%)	0.684 ± 0.363 (+26%)
1200 ppm positive control (sodium phenobarbital)	1.012 ± 0.474** (+52%)	1.460 ± 1.086 (-14%)	1.068 ± 0.756 (+41%)	2.588 ± 1.910** (+74)	1.907 ± 1.102 (+49%)	NA

[a] Data obtained from pages 648 & 654 in the study report (MRID 4980419).
* Statistically different ($p < 0.01$) from the control (Dunnett 2-sided test).
** Statistically different ($p < 0.05$) from the control (Dunnett 2-sided test).
NA= data not available
Percent changes are presented in parentheses and were calculated by the reviewer.

TABLE 36. RIA TSH Group Mean Concentrations [a]

Dose rate ppm	TSH Mean (ng/mL)					
	Day 2	Day 4	Day 8	Day 15	Day 29	Day 89
0	0.752 ± 0.428	1.220 ± 0.592	0.769 ± 0.470	0.770 ± 0.354	0.875 ± 0.615	0.669 ± 0.572
1200 ppm sedaxane	1.111 ± 0.378** (+48%)	1.091 ± 0.386 (-11%)	1.112 ± 0.601 (+45%)	1.504 ± 1.638 (+93%)	1.076 ± 0.779 (+23%)	NA
3600 ppm sedaxane	0.831 ± 0.322 (+11%)	0.889 ± 0.391 (-27%)	0.787 ± 0.307 (+2.3%)	0.972 ± 0.358 (+26%)	1.175 ± 0.630 (+34%)	0.856 ± 0.498 (+28%)
1200 ppm positive control (sodium phenobarbital)	5.365 ± 0.413* (+613%)	8.887 ± 0.589* (+628%)	8.936 ± 0.640* (+1062%)	9.212 ± 1.208* (+1096%)	7.926 ± 0.716* (+806%)	NA

[a] Data obtained from pages 648 & 654 in the study report (MRID 4980419).
* Statistically different ($p < 0.01$) from the control (Dunnett 2-sided test).
** Statistically different ($p < 0.05$) from the control (Dunnett 2-sided test).
NA= data not available; Percent changes are presented in parentheses and were calculated by the reviewer.

CARC concluded that there is a lack of evidence of a clear increase in TSH or decrease in T_3/T_4 levels (Key Events 3 and 4 for the mode of action hypothesis presented in Figure 8). It is noted, however, that shifts in thyroid hormone concentrations can be difficult to capture particularly in cases where the magnitude of effect may be modest (as indicated here by weak thyroid tumorigenic effect).

e) Thyroid follicular cell hypertrophy and increased thyroid weight in sedaxane-treated rats (Associative Event 2)

28 Day Oral (Dietary) Mechanistic Study to Evaluate Effects on the Liver and Thyroid on the Male Rat (MRID 4984819)

Study conditions have been described earlier. Thyroid weights (**Table 37**) were significantly ($p<0.01$) increased on day 29 at both 1200 and 3600 ppm sedaxane. At the end of the treatment-free period, thyroid weights in animals previously treated at 3600 ppm sedaxane were similar to controls indicating the thyroid weight increases were fully reversible. In animals given 1200 ppm sodium phenobarbital, thyroid weights were elevated on days 2, 15 and 29 but not on days 4 or 8.

TABLE 37. Thyroid Weights (Mean ± SD) [a]

	Day 2			Day 4			Day 8		
	Thyroid (g)	**Adjusted (g)**	**% Body Weight**	**Thyroid (g)**	**Adjusted (g)**	**% Body Weight**	**Thyroid (g)**	**Adjusted (g)**	**% Body Weight**
0	0.014 ± 0.002	0.014	0.007 ± 0.001	0.018 ± 0.002	0.018	0.008 ± 0.001	0.015 ± 0.003	0.015	0.007 ± 0.001
1200 ppm sedaxane	0.014 ± 0.003	0.014	0.007 ± 0.002	0.017 ± 0.003	0.017	0.007 ± 0.001	0.014 ± 0.002	0.014	0.006 ± 0.001
3600 ppm sedaxane	0.015 ± 0.002	0.015	0.008 ± 0.001	0.018 ± 0.003	0.019	0.008 ± 0.001	0.015 ± 0.003	0.015	0.007 ± 0.001
1200 ppm positive control (sodium phenobar bital)	0.016 ± 0.002*	0.016**	0.008 ± 0.001	0.018 ± 0.003	0.018	0.007 ± 0.001	0.016 ± 0.003	0.016	0.007 ± 0.001

[a] Data obtained from pages 88-98 in the study report (MRID 4980419).
* Statistically different ($p < 0.01$) from the control (Dunnett 2-sided test).
** Statistically different ($p < 0.05$) from the control (Dunnett 2-sided test).
NA= data not available

TABLE 37 (continued). Thyroid Weights (Mean ± SD) [a]

	Day 15			Day 29			Day 89		
	Thyroid (g)	**Adjusted (g)**	**% Body Weight**	**Thyroid (g)**	**Adjusted (g)**	**% Body Weight**	**Thyroid (g)**	**Adjusted (g)**	**% Body Weight**
0	0.026 ± 0.003	0.026	0.009 ± 0.002	0.014 ± 0.002	0.014	0.005 ± 0.001	0.021 ± 0.003	0.021	0.006 ± 0.001
1200 ppm sedaxane	0.026 ± 0.004	0.026	0.009 ± 0.001	0.018 ± 0.003*	0.018* (+29%)	0.006 ± 0.001	NA	NA	NA
3600 ppm sedaxane	0.026 ± 0.002	0.026	0.010 ± 0.001	0.017 ± 0.003*	0.018* (+29%)	0.006 ± 0.001	0.020 ± 0.003	0.020	0.005 ± 0.001
1200 ppm positive control (sodium phenobar bital)	0.029 ± 0.002*	0.029*	0.010 ± 0.001	0.025 ± 0.004*	0.025* (+79%)	0.008 ± 0.001	NA	NA	NA

[a] Data obtained from pages 88-98 in the study report (MRID 4980419).
* Statistically different ($p < 0.01$) from the control (Dunnett 2-sided test).
** Statistically different ($p < 0.05$) from the control (Dunnett 2-sided test).
NA= data not available

Microscopic pathology revealed no treatment-related thyroid effects on days 2, 4 or 8. On day 15, changes were seen in the thyroid glands (epithelial hypertrophy). This was seen in

one and two animals respectively in the sedaxane-treated animals, and eight in the positive control group (**Table 38**). On day 29, changes seen in the thyroid glands (epithelial hypertrophy) had progressed further in the positive controls and to a lesser extent in the group given 3600 ppm sedaxane. This was also seen in one animal in the group given 1200 ppm sedaxane. On day 89, the animals previously given 3600 ppm of sedaxane and controls were examined after a 60-day treatment free period. After this recovery period, there were no findings that were considered to be related to treatment.

TABLE 38: Microscopic thyroid findings

	Sedaxane			NaPB
	0 ppm	**1200 ppm**	**3600 ppm**	**1200 ppm (0 ppm)[a]**
Thyroid: Follicular cell hypertrophy (N)[b]	(15)	(15)	(15)	(15)
Day 2	0	0	0	0
Day 4	0	0	0	0
Day 8	0	0	0	0
Day 15	0	1 (minimal)	2 (minimal)	8** (6 minimal & 2 slight)
Day 29	0	1 (minimal)	4 (3 minimal & 1 slight)	14** (3 minimal, 9 slight & 2 moderate)
Day 29 (+60)	0	NA	0	NA

[a]For NaPB groups, adjustment of thyroid weights for body weight generated a different adjusted control group mean value (shown in parentheses).

[b]For thyroid follicular cell hypertrophy, range of severities observed are shown in parenthesis. In the study report (Chubb, 2015), the terminology "epithelial hypertrophy" was used, which is synonymous with the term "follicular cell hypertrophy".
NA – Not applicable
*, ** Statistically-significantly different from control with $p<0.05$ and $p<0.01$ respectively. Data from Chubb (2015). (MRID 4980419).

CARC concluded that sedaxane treatment leads to increased thyroid weight at Day 29 (at 1200 and 3600 ppm) and follicular cell hypertrophy at 3600 ppm in the rat (Associative Event 2 for the mode of action hypothesis presented in Figure 8).

f) Additional subchronic toxicity studies from the sedaxane database in Han Wistar rats (CrL:WI(Han)) (Associative Events 1 & 2)

90-Day Oral Study (MRID 47473376)

Han Wistar rats (strain designation CrL:WI(Han)), groups of 10 male and 10 female, were treated with sedaxane at dietary inclusion levels of 0, 300, 2000 and 4000 ppm (Shearer, 2009)

(MRID 47473376). This study was conducted in the same laboratory and strain of rat as the 2-year rat study with sedaxane. Only a limited number of parameters associated with the proposed MOA (liver and thyroid weights and histopathology) were assessed in this study. These data for the male rats are summarized in **Table 39**.

Liver weights adjusted for body weight were statistically significantly higher than control values for 2000 and 4000 ppm males. Thyroid weights showed statistical significance at 300 ppm and 4000 ppm. However, the mean value at 2000 ppm was virtually identical to the control values. In the absence of a dose-related response and considering that all mean values were within the historical control range for both absolute weight and relative weight, these differences in thyroid weights reflect normal variability in a relatively small organ weight, and they do not represent a treatment related effect.

Histopathology findings in the liver including centrilobular hypertrophy and hepatocyte pigment were only increased in the 4000 ppm males. Thyroid micropathology findings consisted of follicular cell hypertrophy (minimal to mild) and were only observed at 4000 ppm.

TABLE 39: Summary of data from a 90-day study with sedaxane (Liver and thyroid-related parameters from male rats only)

	0 ppm	300 ppm	2000 ppm	4000 ppm
Organ Weights:				
Liver wt. adjusted for body weight (g)	15.38	15.71	17.73**	20.94**
Thyroid wt. adjusted for body weight (g)	0.0259	0.0177**	0.0261	0.0210**
Histopathology: (N)	(10)	(10)	(10)	(10)
Liver:				
Centrilobular hypertrophy (mild - moderate)	0	0	0	10***
Hepatocyte pigment (minimal - mild)	0	1	0	4
Thyroid:				
Follicular cell hypertrophy (minimal – moderate)	0	0	0	5*

*, ** and ***: Statistically-significantly different from control with $p<0.05$, $p<0.01$ and $p<0.001$, respectively. Data from (Shearer, 2009) (MRID 47473376).

CARC concluded that sedaxane treatment leads to increased liver weight (at 2000 and 4000 ppm), centrilobular hypertrophy (at 4000 ppm), and follicular cell hypertrophy (at 4000 ppm) in the rat (Associative events 1 and 2 for the mode of action hypothesis presented in Figure 8).

g) Additional subchronic toxicity studies from the sedaxane database in Han Wistar rats (HsdRccHan:WIST) (Associative Event 1)

28-Day (MRID 47473372) and 90-Day Oral Study (MRID 47473375)

A 28-day study and a 90-day study with sedaxane or its isomers were conducted in a different strain of Han Wistar rats (designation: HsdRccHan:WIST). These studies were also conducted in a different laboratory (Syngenta Central Toxicology Laboratory, UK) than the 2-year rat study with sedaxane. In both of these studies, liver weight increases were observed at dose levels of ≥1000 ppm in the diet, and microscopic findings of centrilobular hypertrophy and increased pigmentation in the liver were observed at dose levels of ≥2000 ppm in the diet in male rats. However, no treatment-related changes in thyroid weights or microscopic findings in the thyroid were observed in either of these studies. In addition, no effects on serum levels of thyroid hormones or TSH were observed at the end of the 28-day study (Noakes, 2007 (MRID 47473375); Peffer and Noakes, 2010 (MRID 47473372)).

Based on the results of these studies, the HsdRccHan:WIST rat strain supplied by Harlan Laboratories did not suggest the thyroid changes that were seen in the CrL:WI(Han) supplied by Charles River Laboratories. However, both strains showed similar responses in the liver after dietary treatment with sedaxane.

CARC concluded that sedaxane treatment leads to increased liver weight (at ≥1000 ppm) and centrilobular hypertrophy (at ≥2000 ppm) in the rat (Associative Event 1 for the mode of action hypothesis presented in Figure 8). No effects on thyroid weight or histopathology were observed.

h) Non-neoplastic findings in a combined chronic toxicity and carcinogenicity study in Han Wistar male rats (Associative Events 1 & 2 and Key Event 5)

In addition to the tumor incidence data described in Section VII.A relevant toxicity data in the liver and thyroid generated in the combined chronic toxicity and carcinogenicity study (MRID 47473386) in rats is presented in **Table 40** and **Table 41**. After 52 weeks, dose-responsive increases in liver weights adjusted for body weight were observed at ≥1200 ppm, whereas the liver micropathology findings of centrilobular hypertrophy and hepatocyte pigment occurred only at 3600 ppm in male rats. Thyroid weights were not measured, but the thyroid micropathology finding of follicular cell hypertrophy was increased at ≥1200 ppm (similar to the findings in a 28-day (MRID 4984819) and a 90-day study (MRID 47473376)).

In the carcinogenicity phase of the study, terminal sacrifice animals at 104 weeks (plus early decedents) displayed increased liver weights and increased hepatocellular hypertrophy in males at ≥1200 ppm. In the thyroid, follicular cell hyperplasia was increased only at the high dose (3600 ppm) after 104 weeks; this response was considered an indicator of increased cell proliferation, and is a causal key event in the MOA (**Figure 8**). Colloid basophilia was also increased in the 3600 ppm males at 104 weeks. The other reported micropathology incidences in the male thyroid shown in **Table 41** were either marginally different from controls at the higher dose levels, or represented a decrease in incidence at 3600 ppm and were thus of limited biological relevance.

TABLE 40: Summary of data from 52-week interim sacrifice in 2-year rat study with sedaxane (Non-neoplastic liver- and thyroid-related parameters from male rats only)

		0 ppm	200 ppm	1200 ppm	3600 ppm
Organ Weights:	**Week**				
Liver wt. adjusted for body weight (g)	52	16.10	16.03	18.94**	22.63**
Micropathology: (N)		(12)	(12)	(12)	(12)
Liver:					
Centrilobular hypertrophy	52	0	0	0	11***
Hepatocyte pigment	52	0	1	0	7**
Thyroid:					
Follicular cell hypertrophy	52	0	0	5*, +	4+

*, ** and ***: Statistically-significantly different from control with p<0.05, p<0.01 and p<0.001, respectively. (Dunnett's test or Fisher's Exact Test).
+p<0.05, Mann-Whitney U-test. Data from (Perry, 2010b) (MRID 47473386).

TABLE 41: Summary of data from 104-week sacrifice + decedents in 2-year rat study with sedaxane (Non-neoplastic liver- and thyroid-related parameters from male rats only)

		0 ppm	200 ppm	1200 ppm	3600 ppm
Organ Weights:	**Week**				
Liver wt. adjusted for body weight (g)	104	18.10	18.06	20.21**	24.20**
Micropathology: (N)		(52)	(52)	(52)	(52)
Liver:					
Centrilobular hypertrophy	104	0	0	8**	16***
Hepatocyte pigment	104	0	1	0	1
Thyroid:					
Desquamation, epithelial follicular	104	7	8	11	16
Basophilia, colloid	104	7	9	12	16+
Diffuse C-cell hyperplasia	104	27	27	24	10***
Focal follicular cell hyperplasia	104	7	8	8	16+

*, ** and ***: Statistically-significantly different from control with p<0.05, p<0.01 and p<0.001, respectively. (Dunnett's test or Fisher's Exact Test).
+p<0.05, Mann-Whitney U-test. Data from (Perry, 2010b) (MRID 47473386).

CARC concluded that sedaxane treatment leads to increased liver weight, liver and thyroid hypertrophy at 1200 and 3600 ppm (Associative Events 1 and 2), and thyroid follicular cell

hyperplasia at 3600 ppm in the rat (Key Event 5) for the mode of action hypothesis presented in Figure 8.

VIII. APPLICATION OF THE CANCER GUIDELINES MODE OF ACTION (MOA) FRAMEWORK FOR THYROID TUMORS

A. Postulated MOA and Key Events:

The following narrative were extracted from the registrant submitted MOA and human relevance framework document for rat thyroid tumors (MRID 49804818).

Based on the available information, the registrant's representatives postulated that the MOA for sedaxane-induced rat thyroid tumors is **activation of CAR/PXR and induction of hepatic UGT, which results in a series of downstream events.**

Key events for this MOA include the following:
CAR/PXR activation
Induction of hepatic UGT activity
Reduced circulating T_3 and T_4
Increased circulating TSH
Increased thyroid follicular cell proliferation (hyperplasia)
Increase in thyroid tumor incidence compared to concurrent controls

Associative events for this MOA include the following:
Hepatocellular hypertrophy and increased liver weight
Thyroid follicular cell hypertrophy and increased thyroid weight

B. Dose-concordance of key events

From consideration of the incidence of thyroid follicular cell adenomas and carcinomas in male Han Wistar rats (**Table 30**), 200 and 1200 ppm can be considered non-tumorigenic doses. At 3600 ppm the combined incidence of thyroid adenomas and carcinomas was numerically higher than concurrent controls but not statistically significant using Fisher's Exact Test and was within the range of spontaneous tumor incidence for the test strain as indicated by the RITA data base. The incidence was statistically significant only when evaluated by a Peto trend test (2-sided) on the combined incidence, and the dose level of 3600 ppm is considered the tumorigenic dose for thyroid tumors in male rats. In the studies described in this Assessment, data was generated at a number of different dose levels from 200 ppm to 4000 ppm. **Table 42** describes the registrant proposed key events observed at each dose level.

TABLE 42: Summary of Dose-Concordance of Associative Events and (Causal) Key Events

Dietary inclusion level of Sedaxane (ppm) [a]	**CAR/PXR activation (Causal)**	**Hepatic UDPGT induction (Causal)**	**Increased hepatocellular hypertrophy and/or liver weight (Associative)**	**Reduced circulating total T_3/T_4 (Causal)**	**Increased circulating TSH (Causal)**	**Increased thyroid follicular cell hypertrophy (Associative)**	**Increased thyroid weight (Associative)**	**Increased thyroid follicular cell proliferation and hyperplasia (Causal)**	**Higher Incidence of Combined Thyroid Adenoma + Carcinoma (EPA) (Outcome)**
200 (300) [a]	No data	No data	No	No data	No data	No	No	No	No
1200	Yes[b]	Yes	Yes	Yes	Yes[c]	Yes	Yes (28 days)	No	No
(2000) [a]	Yes[d]	Yes[d]	Yes	Yes[d]	Yes[c,d]	Yes	No (90 days)	No data	No data
3600 (4000) [a]	Yes[b]	Yes	Yes	Yes	Yes[c]	Yes	Yes (28 days)	Yes	Yes

[a] Values in parentheses are subchronic dose levels (that are similar to the chronic dose levels, or in between the chronic dose levels).

[b] CAR/PXR activation was suggested based on *in vitro* studies, and by increased PROD activity (a marker of Cyp2b activity) in the livers of rats on Day 8 of the 28-day MOA study at the indicated dose levels (Chubb, 2015) (MRID 4984819).

[c] TSH levels were numerically higher than the control values on Days 15 and 29, the same time points that NaPB produced maximal increases in TSH, and correlated with a return of T3 and T4 levels to the same as (or slightly above) control levels.

[d] Assumed response (not evaluated at 2000 ppm), based on responses at higher and lower dose levels.

Overall, the registrant concluded there is good dose concordance of the proposed key events. The initial causal key event of CAR/PXR activation was assessed based on *in vitro* reporter assays and the surrogate measure of PROD activity in the liver. PROD activity is a marker of Cyp2b induction, an isoform that is characteristic of CAR/PXR activation, and this activity was increased in a dose-responsive manner at 1200 and 3600 ppm sedaxane. The large increase in PROD activity (e.g. 69-fold at 3600 ppm sedaxane) is very characteristic of CAR activators, including NaPB. Similarly, the causal key event of UDPGT induction (with thyroxine as substrate) was also increased in a dose-responsive manner at both 1200 and 3600 ppm. As a consequence of this UDPGT induction, the registrant concluded that increased clearance of T_4 led to decreased T_3 and T_4 levels at 1200 and 3600 ppm at early time points. Later (Days 15-29), a marginal increase in TSH was observed at these same dose levels. While all of these early effects could be indicated in a dose-responsive manner at both 1200 ppm and 3600 ppm, the later key event of hyperplasia was only observed at 3600 ppm. The combined thyroid tumor incidence at 3600 pm, although numerically higher, was only statistically significant using the Peto Trend test with no statistical significance attained using Fisher's Exact Test.

For the associative events, the expected increases in liver centrilobular hypertrophy and weight were observed in a dose-responsive manner consistent with the proposed MOA. These liver parameters were affected consistently across multiple studies, and they occurred at dose levels at or below the tumorigenic dose level of 3600 ppm. Thyroid follicular cell hypertrophy was observed with low severity and partial incidence at the higher doses of 1200 and 3600 ppm only, but not at the low dose of 200 ppm (1-year and 2-year time points) or 300 ppm (90-day study). Increases in thyroid weight were observed at only selected early time intervals at 1200 and 3600 ppm (Day 29), but not after 90 days of treatment. Overall, the registrant concluded that associative events in the liver were clearly and consistently observed across multiple studies, and the associative events in the thyroid after sedaxane treatment were affected in multiple studies (but to a lesser extent than the liver effects).

C. Temporal-concordance of key events

When the tumorigenic and non-tumorigenic dose levels are considered, the registrant concluded that the observed effects on parameters associated with the key events occurred in a logical, time-dependent manner consistent with the proposed MOA. The temporal-concordance is summarized in **Table 43**.

The observed steps occur in a logical sequence, with the key events preceding the time of occurrence of thyroid follicular adenomas. In particular:

- CAR/PXR activation, induction of hepatic UDPGT, elevated total P450 and microsomal enzyme activities, increased liver hypertrophy and increased liver weight occurred early (within 1-7 days) and remained consistently affected over time
- Decreases in circulating T_3/T_4 occurred early (within 1-7 days), and these values had returned to control levels by the last time of measurement (28 days)
- TSH was unaffected after 1-7 days, but showed a marginal increase at 14-28 days, reflecting a response of the HPT axis to lower T_3/T_4 levels

- Thyroid follicular cell hypertrophy was not apparent at 1-7 days, but it was observed at 14 days up through 1 year. This was consistent with increased stimulation of the thyroid by increased TSH levels.
- After >1-2 years, increased thyroid follicular hyperplasia (as an indicator of increased thyroid follicular cell proliferation) was observed.
- A higher incidence of adenomas plus carcinomas of the thyroid required >1-2 years before it was observed.

TABLE 43: Summary of Temporal Concordance of Associative and (Causal) Key Events

Time[c]	CAR/PXR Activation and Hepatic UDPGT induction (Causal) [a]	Increased hepatocellular hypertrophy and liver weight (Associative)	Reduced circulating T_3/T_4 (Causal)	Increased circulating TSH (Causal)	Increased thyroid follicular cell hypertrophy (Associative)	Increased thyroid weight (Associative)	Increased thyroid follicular cell proliferation and hyperplasia (Causal)	Higher Incidence of Combined Thyroid Adenoma + Carcinoma (EPA)
1-7 days	Yes	Yes	Yes	No	No	No	No	No
14 days	Yes	Yes	Yes	Yes[b]	Yes	No	No	No
28 days	Yes	Yes	No	Yes[b]	Yes	Yes	No	No
90 days	No data	Yes	No data	No data	Yes	Yes	No	No
1 year	No data	Yes	No data	No data	Yes	No data	No	No
>1 - 2 years	No data	Yes	No data	No data	No	No data	Yes	Yes

[a]CAR/PXR activation was suggested based on *in vitro* studies, and by increased PROD activity (a marker of Cyp2b activity) in the livers of rats on Day 8 of the 28-day MOA study at the indicated dose levels (Chubb, 2015) (MRID 4984819).

[b]In the mode of action study, TSH levels were numerically higher than the control values on Days 15 and 29, and correlated with a return of T3 and T4 levels to the same as (or slightly above) control levels

[c]Time of continuous treatment – as opposed to the Study Day (e.g. Day 2, Day 4, Day 8), which is 1 day longer by convention.

D. Reproducibility and consistency

Where parameters were measured in multiple studies, the registrant concluded that there is a high degree of reproducibility between studies and consistency between key events. The first key causal event in the proposed MOA, activation of CAR/PXR in the liver, was confirmed both by *in vitro* reporter assays and by increases in Cyp2b isoenzymes (PROD activity) in the liver *in vivo*. The causal key event of induction of hepatic UDPGT showed a high degree of consistency across multiple time points in the 28-day mechanistic study. Similarly, the associative events of hepatomegaly and hepatocellular hypertrophy were observed in every study in the rat. Thyroid hormone measurements were only determined in this strain of rat in a single study so their reproducibility between studies cannot be assessed. Thyroid follicular cell hypertrophy was seen in the 28-day MOA study, the 90-day study and at the 1-year sacrifice of the chronic toxicity/carcinogenicity study in rats.

In studies involving 28 or 90 days treatment with sedaxane, the HsdRccHan:WIST rat strain supplied by Harlan Laboratories did not suggest the thyroid changes that were seen in the CrL:WI(Han) supplied by Charles River Laboratories. However, both strains showed similar responses in the liver after dietary treatment with sedaxane. These differences are considered a reflection of possible strain differences in sensitivity for this thyroid MOA, and the definitive data to investigate the key events were produced in studies with the CrL:WI(Han) strain of rat, the same strain that was used in the 2-year chronic/carcinogenicity study.

E. Biological Plausibility

The induction of thyroid follicular cell adenomas in rats is a common finding in chronic toxicity and carcinogenicity studies (Finch *et al.*, 2006; Hurley, 1998; Wilson *et al.*, 1996). The proposed MOA (**Figure 8**) for the thyroid effects seen with sedaxane, which can be described as a perturbation of the HPT axis secondary to induction of hepatic UDPGT, is well described for a number of compounds, including the archetypal UDPGT inducer, NaPB (Finch *et al.*, 2006) and a number of SDHI fungicides including benzovindiflupyr (EPA, 2014b), fluopyram (EPA, 2014a) and fluxapyroxad (EPA, 2011a).

F. Alterative mode of action hypotheses

In addition to the MOA described (**Figure 8**), alternative modes of action for the induction of thyroid tumors were suggested by the registrant. One such alternative MOA is genotoxicity. This MOA can be excluded as sedaxane has been demonstrated to be negative for genotoxicity in a comprehensive package of *in vitro* and *in vivo* assays for genotoxicity (**Table 28**).

A second alternative MOA is direct inhibition of the thyroid hormone synthesis. Organification of iodine *via* monoiodination of L-tyrosine is the first step in the synthesis of T_3 and T_4 and is catalyzed by the enzyme thyroid peroxidase (TPO). Inhibition of TPO, in order to reduce circulating T_3/T_4, by compounds such as propylthiouracil (PTU) is exploited as a treatment for hyperthyroidism in humans, such as in Graves' disease. PTU has also been shown to induce thyroid follicular cell adenomas in rats (IARC, 2001). This MOA can be excluded for sedaxane

as it was found not to be an inhibitor of male rat thyroid-derived TPO *in vitro*, whereas PTU was shown to be a potent inhibitor (Lake, 2014) (MRID 49804821).

G. Uncertainties, inconsistencies and data gaps

The registrant concluded that the available data support the proposed hypothesized MOA for the slightly increased incidence of rat thyroid tumors by sedaxane (**Figure 8**), while excluding the alternative MOAs described above. Only minor uncertainties and no data gaps remain.

The registrant concluded that TSH levels were numerically higher than controls on Days 15 and 29 of treatment, which matched the time points when the effect of NaPB on TSH was maximal, but these differences in the sedaxane groups were not statistically significant at the time intervals that were assessed in this study. Considering the weaker response for most key events with sedaxane treatment, relative to the responses with NaPB treatment, a marginal effect on TSH levels is not unexpected. The timing of the increases in TSH levels with compounds that produce thyroid effects *via* induction of UDGPT in the liver can vary by compound and rat strain, such that TSH increases following sedaxane treatment might have been maximal during a time window that was not among the time points selected for this sedaxane MOA study.

The hypothesized consequence of UDPGT induction, increased clearance of T_3/T_4 from the blood into the bile, has not been directly demonstrated; however, the consequence of this increased clearance, namely decreased T_3/T_4 in the serum was suggested and was associated with the increased hepatic UDPGT. Therefore, the registrant concluded that it is reasonable to infer that all of the intermediate key events are operating.

IX. ARE THE KEY EVENTS IN THE ANIMAL MODE OF ACTION FOR THYROID TUMORS PLAUSIBLE IN HUMANS?

Following establishment of a plausible MOA for the induction of thyroid tumors in rats, the next step is to assess the relevance to humans by assessing the qualitative and quantitative differences between the rat and human for each of the key events.

In contrast to rats, serum TSH levels in humans are more stable following exposure to hepatic enzyme inducers (Dellarco *et al.*, 2006; Meek *et al.*, 2003). The human HPT axis is qualitatively very similar to that of rats and it has been demonstrated that human administration of pharmaceuticals that result in the induction of UDPGT, including phenobarbital, phenytoin and carbamazepine also result in reduced circulating T_3/T_4.

However, despite the reduced T_3/T_4 levels, TSH levels in humans remain largely unaffected, whereas in the rat TSH levels increase in order to compensate (Curran and DeGroot, 1991). Therefore, although the HPT axis is responsible for homeostatic control of thyroid hormones in both species, there is a large difference in their sensitivity to perturbation, with the human being considerably less susceptible (Dellarco *et al.*, 2006).

In addition to differential sensitivity of the HPT axis, another factor resulting in lower sensitivity of humans as compared to rats is that the half-life of T_3 and T_4 in humans is considerably longer than that in rats, being 5-9 days in humans and 12 hours in rats for T_4 (Dohler *et al.*, 1979; U.S. Environmental Protection Agency, 1998). The substantially longer half-life in humans is a result of binding to a high-affinity thyroid-binding globulin, which binds T_4 (and T_3 to a lesser degree), and is not present in rats (Hill *et al.*, 1998; U.S. Environmental Protection Agency, 1998). These differences mean that rats have a higher rate of turnover of T_3/T_4. As a result of this higher turnover, rats have a much higher (approximately 25-fold) basal level of TSH when compared to humans (Dohler *et al.*, 1979). This means that the compensatory reaction in rats towards a T_3/T_4 deficiency is much more pronounced than in humans.

Finally, it has been suggested that interspecies differences in thyroid histology play a role in the differential sensitivity. In humans, the thyroid follicular cell epithelium is composed of short, cuboidal cells, indicative of their quiescent nature. In rats, however, the thyroid follicular cells are tall and cuboidal and appear to be continually active in synthesis. Therefore, it appears that the rodent thyroid gland is chronically stimulated by TSH levels to compensate for the increased clearance of thyroid hormones. It follows that increases in TSH levels above basal levels in rats more readily moves that gland towards increased growth and potential neoplastic change than in humans (Dellarco *et al.*, 2006; U.S. Environmental Protection Agency, 1998). Interestingly, adult male rats have higher serum TSH levels than female rats (Chen, 1984), and they are often more sensitive to stimulation of thyroid growth and carcinogenesis. Overall, the histological differences in thyroid follicular cells between rats and humans is related to a higher rate of production of T_4 in rats to maintain a consistent serum concentration, thus making the rat thyroid more "functionally active" than primates including humans (Dellarco *et al.*, 2006; McClain, 1995).

Even though certain agents can cause a reduction in T_3/T_4 levels in humans, there is no evidence that these agents can induce an increased susceptibility to thyroid cancer in humans (Dellarco *et al.*, 2006; Ron *et al.*, 1987). Epidemiology studies with phenobarbital have not shown any increased risk for thyroid cancer in humans (Olsen *et al.*, 1993). As a result, the only known human thyroid carcinogen is radiation, which is a mutagenic mode of action.

In summary, a wealth of information in the literature has established a lack of susceptibility of humans to thyroid hormone alterations, resulting changes in TSH, and thyroid tumor responses that are initiated in rats by induction of UDPGT and increased T_3/T_4 clearance. Therefore, based on qualitative differences (presence of high-affinity thyroid binding globulin in humans but not rodents) and quantitative differences (including lower responsiveness to fluctuations in thyroid hormone levels), the MOA established in rats with sedaxane is not relevant to humans.

X. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE FOR THE MOA FOR THYROID TUMORS IN MALE RATS

On February 22, 2017, CARC reconvened to evaluate the submissions of new studies containing MOA data on sedaxane. From these deliberations, the CARC drew the following conclusions for the sedaxane-induced rat thyroid tumor MOA:

- **There is evidence that sedaxane causes direct activation of CAR (mouse, rat, and human) and PXR (rat and human) nuclear receptors as evidenced by *in vitro* gene expression. There is *in vivo* data supporting an increase in microsomal protein content (rat), UGT activity (rat), total CYP content (rat and mouse), PROD activity (rat and mouse), testosterone 16β-hydroxylase activity (rat), and testosterone 6β-hydroxylase activity (rat and mouse). RT-PCR data presented an increase in Gadd45β mRNA levels (mouse). In addition, microarray data showed an increase in expression of xenobiotic metabolizing enzymes and other genes associated with CAR/PXR activation (mouse).**

- **There is evidence of increased liver weight and increased hepatocellular hypertrophy.**

- **There is a lack of evidence of a clear increase in TSH and decrease in T_3/T_4 levels. It is noted that shifts in thyroid hormone concentrations can be difficult to capture particularly given the weak evidence of thyroid tumors in male rats.**

- **There is evidence of increased thyroid weight, follicular cell hypertrophy and hyperplasia.**

- **There is good concordance between the dose causing tumors (3600 ppm male rats) and the dose response and temporal associations for the key and associative events.**

- **Alternative MOAs (*i.e.,* genotoxicity and direct inhibition of the thyroid hormone synthesis) have been adequately ruled out.**

Conclusion: There is plausible evidence that the mode of action (MOA) for the rat thyroid tumor induced by sedaxane is CAR-mediated induction of hepatic UGT activity, leading to decreased circulating T3/T4, increased TSH and thyroid follicular cell proliferation.

XI. EVALUATION OF UTERINE TUMORS AND MECHANASTIC STUDIES

As stated above, CARC considered the uterine tumors to be treatment-related in female rats, based on the following information:

A. Uterine Tumors in Female Rats:

*The following text in Section XI. A-C was extracted directly from the first CARC meeting (March 16, 2011) report (TXR #0055706)**.***

In a combined chronic toxicity/carcinogenicity study, 52 Crl:WI(Han)(Han Wistar) rats/sex/dose were exposed to sedaxane (95.3% a.i.) for up to 2 years in the diet at concentrations of 0, 200, 1200, or 3600 ppm (equivalent to 0/0, 11/14, 67/86, and 218/261 mg/kg bw/day in males/females, respectively) (MRID 47473386). An additional 12 rats/sex/dose were treated similarly for up to 1 year and then sacrificed.

As shown in **Table 44** below, no statistically significant increases were seen for uterine adenomas. Female rats had a statistically significant trend ($p<0.01$), and significant pair-wise comparisons of the 200 ppm ($p<0.05$) and 3600 ppm dose groups ($p<0.01$) with the controls, for uterine adenocarcinomas. There was a statistically significant trend ($p<0.01$) and significant pair-wise comparisons of the 200 ppm ($p<0.05$), 1200 ppm ($p<0.05$) and 3600 ppm ($p<0.01$) dose groups with the controls for combined tumors.

Table 44. Sedaxane – Crl:WI(Han)(Han Wistar) Female Uterine Tumor Rates[+]

	Dose (ppm)			
	0	**200**	**1200**	**3600**
Adenomas	0/44	0/35	1[a]/38	0/44
(%)	(0%)	(0%)	(3%)	(0%)
p =	0.52989	-	0.14095	-
Adenocarcinomas	0/50	3/43	2/44	9[b]/49
(%)	(0%)	(7%)	(5%)	(18%)
p =	0.00033**	0.02457*	0.08172	0.00080**
Combined	0/50	3/43	3/44	9/49
(%)	(0%)	(7%)	(7%)	(18%)
p =	0.00053**	0.02457*	0.03866*	0.00080**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First adenoma observed at final sacrifice, dose 1200 ppm.
[b]First carcinoma observed at week 89, dose 3600 ppm.

Note: Significance of trend denoted at control (the p values on the control groups are the trends).
Significance of pair-wise comparison with control (dose 0) denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

The historical control data for the testing laboratory and the RITA database are listed below. It should be noted that while no adenocarcinomas were seen in the concurrent controls in this study, the laboratory historical control data from 5 studies shows a mean of 10.4% with a range of 0-19% for this tumor type. The range for adenocarcinomas was 0-28% in the RITA database. It is also noted that the combined tumor incidence in the uncensored animals (0%) was lower than the historical control range for the conducting laboratory (2-22%) but within the RITA database historical control range (0-30%).

	Historic Control Data - Range	
	Lab (CRL)	RITA
Adenomas	0-4%	0-6%
Adenocarcinomas	0-19%	0-28%
Combined	2-22%	0-30%

Lab (CRL) data refers to 5 prior or concurrent studies at CRL in 2005-2009

RITA data refers to 38 studies conducted in the Wistar rat from 1997-2006.

Historical control data for each tumor type for 5 studies from the testing laboratory (CRL) are as follows:

Tumor Type	**Range**	**Mean**	**SD**
Adenomas	0-4%	2.8%	1.8%
Adenocarcinomas	0-19%	10.4%	7.0%
Total tumors	2-22%	12.6%	7.2%

B. Other Related Toxic Effects in Female Rats

A decrease in body weight and body weight gain was observed in males at 3600 ppm and in females at ≥1200 ppm. A corresponding decrease in food consumption and food efficiency was observed in both sexes at the high-dose (3600 ppm).

Liver and thyroid findings are discussed elsewhere within this document.

C1. CARC Conclusions on Female Rats:

From these data, the CARC considered the uterine tumors to be treatment-related in female rats.

C2. Additional Discussion on the Uterine Tumorigenic Dose Level

On March 8, 2017, the CARC reconvened to clarify the tumorigenic dose level for uterine tumors. The original CARC report (Kidwell, 2012; TXR #0055706) stated uterine tumors were treatment-related in female rats, however, the dose level(s) considered to be tumorigenic were not explicitly stated.

At 200 and 1200 ppm, the incidences of adenocarcinomas (200 ppm: 7%/1200 ppm: 5%) and combined tumors (200 ppm: 7%/1200 ppm: 7%) are within the historical control range for both the testing lab (Han Wistar rats (Crl:WI(Han)) (adenocarcinomas: 0-19%/combined: 2-22%) and RITA database (Wistar rats) (adenocarcinomas: 0-28%/combined: 0-30%) controls. It is also noted that the combined tumor incidence in the control animals (0%) was lower than the

historical control range for the conducting laboratory (2-22%) but within the RITA database historical control range (0-30%). Historical control data from the testing lab shows that at 200 and 1200 ppm the percentage of adenocarcinomas and combined tumors are below the mean observed (adenocarcinomas: 10.4%/combined: 12.6%) for each tumor type.

It is possible that the statistically significant dose trend that was observed for both adenocarcinomas and combined tumors is driven by the high-dose group; no increase in adenocarcinomas and combined tumors were seen between the low (200 ppm) and mid-doses (1200 ppm). For non-rare tumor types, such as uterine, a more appropriate statistical cut-off is a $p<0.01$ rather than $p<0.05$ in order to reduce the false-positive rate (Haseman, 1983). The significant pair-wise comparison for adenocarcinomas at 200 ppm and combined tumors at 200 ppm and 1200 ppm are significant at $p<0.05$ but not $p<0.01$.

An examination of the EPA database for additional chemicals that observed uterine tumors was conducted to determine if the zero incidence of uterine tumors seen in the sedaxane control animals is a typical occurrence for this tumor type. The table below presents the control animal data for chemicals that noted uterine tumors within Wistar rat carcinogenicity studies. For the two tumor types observed in the sedaxane experiment, adenocarcinomas and combined tumor types, there were no observed incidences of zero uterine tumors in the control animals. In addition, isopyrazam, a structurally and toxicologically similar compound to sedaxane, resulted in increased uterine tumors only at the highest dose tested (3000 ppm).

Chemical name	Adenomas	Adenocarcinomas	Combined
Sedaxane	0/44 (0%)	0/50 (0%)	0/50 (0%)
Isopyrazam	1/63 (2%)	1/63 (2%)	2/63 (2%)
Spirodiclofen	0/26 (0%)	4/47 (9%)	4/47 (9%)
Thiacloprid	0/43 (0%)	6/47 (13%)	6/47 (13%)

Other changes observed only at 3600 ppm include a large decrease in body weight (-33% at week 104), decreased vaginal mucification (**Table 49**), and decreased mammary fibroadenomas (**Table 47**). A decreased incidence of mammary tumors suggests disruption in serum prolactin.

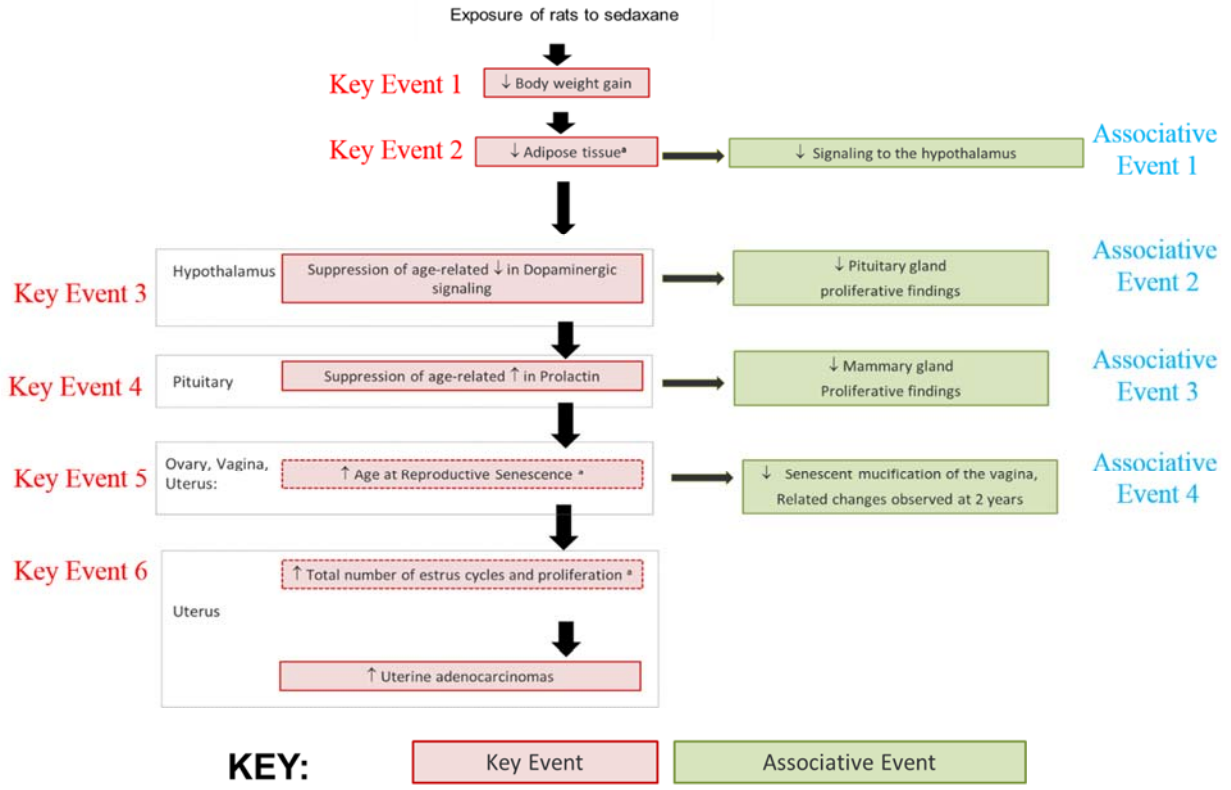
Given the weight-of-the-evidence presented above, the CARC considers 3600 ppm to be the uterine tumorigenic dose level.

D. Registrant's Proposed MOA for Uterine Tumors in Female Rats

A mode of action has been postulated by the Registrant (MRID 49804813) for sedaxane-induced rat uterine tumors. The key events in the proposed MOA include: decreased body weight gain, decreased adipose tissue, suppression of age-related decrease in dopaminergic signaling,

suppression of age-related increase in prolactin, increased age at reproductive senescence, increase in the total number of estrus cycles and proliferation, and an increase in uterine adenocarcinomas (**Figure 9**).

Figure 9: Mode of Action Hypothesis for the Induction of Uterine Tumors in Rats



[a] Key events without sedaxane-specific data that are proposed based on indirect evidence with sedaxane (associative events), and/or known rat biology established in the literature.

2. Sedaxane-specific data to support the proposed MOA

a) Decreased body weight gain in the 2-year chronic carcinogenicity study (MRID 47473386) (Key Event 1)

Mean body weight and body weight gains for female rats are listed in **Table 45**. Females at 3600 ppm showed statistically and biologically significantly lower body weight and body weight gains compared to their respective controls starting at week 1 and continuing throughout the treatment period. The divergence from the control group values were accelerated in the second year of the study, with a greater percent difference from control at 104 weeks (-50%) than at 52 weeks (-34%) for cumulative body weight gain change.

Final body weight and body weight gains throughout the study period of 1200 ppm females were decreased. These body weight and body weight gain effects were considered to be treatment-related. The 200 ppm group did not produce any treatment related changed in body weight or body weight gain. Females treated at 3600 ppm showed statistically significantly lower food

consumption compared to their respective controls throughout the treatment period (data not shown).

TABLE 45: Female body weight and body weight gain from 2-year chronic carcinogenicity study

Mg/kg bw/d	0 ppm	200 ppm	1200 ppm	3600 ppm
Initial BW	129.4 ± 11.4	129.9 ± 9.8	129.9 ± 10.7	132.5 ± 11.1
Week 1	152.7 ± 12.6	152.5 ± 11.1	150.4 ± 12.1	144.9 ± 11.1** (-5)
Week 3	183.4 ± 16.0	185.0 ± 13.1	180.5 ± 12.7	167.9 ± 13.1** (-8)
Week 13	235.5 ± 18.4	238.0 ± 15.8	232.2 ± 16.8	205.4 ± 14.4** (-13)
Week 26	259.0 ± 22.6	259.0 ± 18.0	249.0 ± 18.7** (-4%)	219.4 ± 16.4** (-15)
Week 34	268.3 ± 25.4 (n = 63)	269.4 ± 20.7 (n = 63)	258.6 ± 21.5* (-4%) (n = 63)	228.1 ± 16.9** (-15) (n = 62)
Week 52	292.9 ± 38.7 (n = 63)	295.5 ± 31.7 (n = 62)	281.1 ± 32.6 (n = 63)	240.2 ± 21.2** (-18) (n = 61)
Week 58	311.5±44.5 (n=51)	312.1±32.0 (n=50)	294.9±37.8 (-5) (n=51)	244.5±23.6** (-22) (n=51)
Week 72	345.3±53.0 (n=51)	343.4±40.4 (n=47)	322.5±49.1* (-7) (n=50)	253.4±26.4** (-27) (n=50)
Week 86	380.1±61.5 (n=50)	368.5±46.2 (n=44)	343.8±50.1** (-10) (n=46)	259.9±26.5** (-32) (n=49)
Final BW (Week 104)	392.5 ± 59.1 (n = 44)	389.9 ± 55.3 (n = 35)	362.2 ± 58.1* (-8) (n = 38)	264.1 ± 28.6** (-33) (n = 44)
BWG Week 0-1	23.2 ± 5.7	22.6 ± 5.3	20.5 ± 4.2** (-12)	12.4 ± 4.2** (-47)
BWG Week 0-3	54.0 ± 9.8	55.1 ± 7.6	50.6 ± 8.2 (-6)	35.4 ± 7.6** (-34)
BWG Week 0-52	163.5 ± 33.3	165.6 ± 26.2	151.1 ± 28.5* (-8)	108.1 ± 15.2** (-34)
BWG Week 0-58	180.5±39.0 (n=51)	180.8±28.9 (n=50)	165.4±33.9* (-8%) (n=51)	112.0±18.2** (-38) (n=51)
BWG Week 0-72	214.3±47.9 (n=51)	212.4±38.7 (n=47)	193.1±44.9* (-10%) (n=50)	120.9±21.9** (-44) (n=50)
BWG Week 0-86	249.1±57.6 (n=51)	237.4±45.3 (n=44)	213.4±45.1** (-14%) (n=46)	127.5±22.2** (-49) (n=49)
Overall BWG Week 0-104	262.6 ± 55.8	259.2 ± 53.9	232.7 ± 54.2* (-11)	132.4 ± 23.4** (-50)

[a] Data obtained from pages 82-101 in the study report (MRID 47473386).

* Significantly different from control, $p<0.05$

** Significantly different from the control, $p<0.01$

BW: body weight, BWG: body weight gain, week

Females at 3600 ppm had statistically significantly lower food efficiency during weeks 1-4 and 9-13, and overall for weeks 1-13, in comparison to controls (**Table 46**). This effect indicates that palatability was not a major factor in the decreased body weight and body weight gain observed in the high-dose animals and that the decrease was a treatment-related effect.

TABLE 46: Female food efficiency (mean ± SD) (g/100g weight gain) (% change from controls) [a] from 2-year chronic carcinogenicity study

ppm	0 n = 16	200 n = 16	1200 n = 16	3600 n = 16
Females				
FE Week 1-4	13.7 ± 1.3	13.6 ± 0.8	12.8 ± 1.1* (-7)	10.1 ± 0.8** (-26)
FE Week 5-8	4.8 ± 0.9	5.3 ± 0.8 (+10)	5.4 ± 1.1 (+13)	5.4 ± 1.0 (+13)
FE Week 9-13	2.8 ± 0.5	2.6 ± 0.6 (-7)	2.8 ± 0.6	1.5 ± 0.8** (-46)
Overall FE Week 1-13	6.8 ± 0.5	6.8 ± 0.4	6.6 ± 0.2	5.3 ± 0.4** (-22)

[a] Data extracted from pages 102-107 of the study report (MRID 47473386)
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$
FE: food efficiency

CARC concluded that sedaxane treatment leads to a decrease body weight at 1200 (slight) and 3600 ppm and food efficiency at 3600 ppm in female rats (Key Event 1 for the mode of action hypothesis presented in Figure 9).

b) Decreased adipose tissue in the 2-year chronic carcinogenicity study (MRID 47473386) (Key Event 2)

The following text in Section b was taken directly from the Registrants proposed MOA package (MRID 49804813).

In the 104-week chronic/carcinogenicity study in rats, specific measurements that would reflect a decrease in the percentage of adipose tissue [*e.g.,* percentage (by weight) abdominal fat pads, omental fat] were not a routine part of the study design, and, therefore, a direct measure of adipose tissue in the 3600 ppm female rats was not performed. However, based on known responses in rat studies to caloric restriction, and the increasing percentage of body weight in obese rats that is represented by fat at the end of a 2-year *ad libitum* feeding study, it can be inferred that 3600 ppm sedaxane-treated female rats that were greatly lower than controls in body weight (-33% at 104 weeks) had lower percentages of their body weight as adipose tissue than the controls.

CARC concluded that there is no direct evidence from the registrant submitted studies that sedaxane treatment leads to a decrease in adipose tissue (Key Event 2 for the mode of action hypothesis presented in Figure 9).

c) Shift in tumor profile with sedaxane treatment (Associative Events 2 & 3)

The following text in Section c was taken directly from the Registrants proposed MOA package (MRID 49804813).

Concomitant to the uterine adenocarcinoma incidence, there is a decrease in the incidences of mammary gland fibroadenomas (statistically significant at p<0.001) and anterior pituitary adenomas (not statistically significant; 31% vs. control at 44%) in the 3600 ppm females (**Table 47**). As a further exploration of these associations, both neoplastic and non-neoplastic pathology findings in the mammary gland and pituitary gland are summarized in **Table 48**.

TABLE 47: Incidences of Uterine Endometrial Tumors, Mammary Gland Fibroadenomas, and Pituitary Gland Adenomas in Female Han Wistar Rats at the Conclusion of a 2-Year Carcinogenicity Study – Terminal Sacrifice + Decedents

Table 47A: Sedaxane 2-year study - Selected tumor incidences:

Tumor Type	Dietary inclusion level of Sedaxane in ppm (mg/kg/day)			
	0	200 (14 mg/kg/day)	1200 (86 mg/kg/day)	3600 (261 mg/kg/day)
Uterine				
Adenomas (%)	0/52 (0%)	0/52 (0%)	1/52 (2%)	0/52 (0%)
Adenocarcinomas (%)	0/52 (0%)	3/52 (6%)	2/52 (4%)	9/52** (17%)
Combined (%)	0/52 (0%)	3/52 (6%)	3/52 (6%)	9/52** (17%)
Mammary Gland				
Fibroadenomas (%)	14/52 (27%)	9/50 (18%)	10/51 (20%)	0/52*** (0%)
Pituitary Gland				
Adenoma, Anterior lobe (%)	23/52 (44%)	29/52 (56%)	20/52 (38%)	16/52 (31%)

, * Statistically-significantly different from control with p<0.01, p<0.001 (Fisher's Exact Test) as reported in the original rat 2-year report [mammary gland and pituitary gland (Perry, 2010b) (MRID 47473386)] or in Peffer (2011) (MRID 48534701) (uterus).

Table 47B: Historic control data in Wistar rats (± 5 years from start of sedaxane study)

	Historic Control Data - Range	
Uterine tumors:	Lab (CRL)	RITA
Adenomas	0-6%	0-6%
Adenocarcinomas	0-19%	0-22%
Combined	0-23%	0-28%

Lab (CRL) data refers to 10 prior or concurrent studies at CRL in 2002-2012, ± 5 years from start of sedaxane rat chronic/carcinogenicity study
RITA data refers to 22 studies conducted in the Wistar rat from 2002-2012, ± 5 years from start of sedaxane rat chronic/carcinogenicity study. Registry of Industrial Toxicology Animal Data (RITA), http://reni.item.frauhofer.de/reni.

TABLE 48: Incidence of Pathology Findings in Pituitary and Mammary Gland – Associative Events in the 2-Year Carcinogenicity Study (Female Rats)

	Survivors (104 Weeks)				**Decedents**			
	Dose Groups							
Pathology findings	**0**	**200 pm**	**1200 ppm**	**3600 ppm**	**0**	**200 ppm**	**1200 ppm**	**3600 ppm**
Pituitary Gland	(44)	(35)	(37)	(44)	(8)	(17)	(15)	(8)
No abnormality detected	7	9	8	19**	2	3	6	3
Focal hyperplasia, anterior lobe	14	11	17	9	1	2	0	3
Adenoma, anterior lobe [B]	19	16	15	14	4	13	5	2
Carcinoma, anterior lobe [M]	0	0	0	0	0	0	1	0
Adenoma, intermediate lobe [B]	1	0	0	0	1	0	0	0
Ganglioneuroma [B]	1	0	0	0	0	0	0	0
Mammary Gland	(44)	(34)	(36)	(44)	(8)	(16)	(15)	(8)
No abnormality detected	8	8	7	25***	0	2	5	6**
Fibroadenoma [B]	11	6	7	0***	3	3	3	0
Lobular hyperplasia, with atypia	13	11	7	1***	0	0	0	0
Adenoma [B]	1	0	0	0	1	0	0	0
Adenocarcinoma [M]	3	1	0	0	0	0	1	0

[B] – benign tumor; [M] – malignant tumor. Data from Perry (2010b) (MRID 47473386).
*, **, *** statistically significant by pairwise Fisher's Exact Test ($p<0.05$, 0.01, 0.001)

Examining the mammary gland data, there is a statistically significant decrease in mammary gland fibroadenomas, and the overall data across all groups showed a statistically significant negative trend (Peto trend test; including decedents). In addition, the 3600 ppm females from the 2-year terminal sacrifice, and among the decedent animals, had a statistically significantly higher incidence of "no abnormality detected" in the mammary gland (**Table 48**). In the 3600 ppm group (2-year sacrifice and decedents), there was a zero incidence of mammary fibroadenoma. In addition, the non-neoplastic finding of "lobular hyperplasia with atypia" was statistically significantly lower in the 3600 ppm females at the terminal sacrifice. The registrant concluded

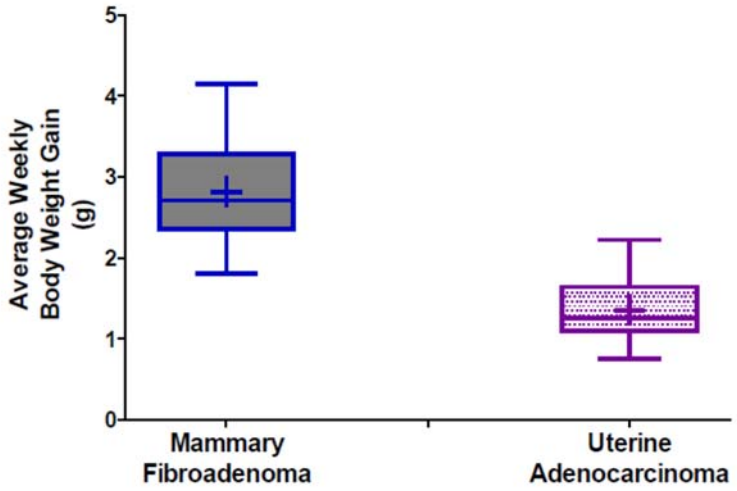
that these incidences are considered an Associative Event that are reflective of the proposed lower levels of circulating prolactin, as mammary fibroadenomas in rats are considered to be responsive to this hormonal input over time (Greaves, 2007; Hargreaves and Harleman, 2011; Keenan *et al.*, 1996).
Examining the pituitary gland data, the 3600 ppm females from the 2-year terminal sacrifice had a statistically significantly higher incidence of "no abnormality detected" in the pituitary gland compared to the controls. Along with this observation, there was a numerically lower incidence of pituitary adenomas (anterior lobe) compared to the control group or the 200 and 1200 ppm groups, and the incidence of this finding in the decedent animals was also lower at 3600 ppm (2/8) than in the control group (4/8). A related non-neoplastic marker of proliferation, focal hyperplasia of the anterior pituitary, was also numerically lower in the 3600 ppm terminal sacrifice animals than in the other control and treated groups. Only the incidence of "no abnormality detected" achieved statistical significance, but a pattern of lower proliferative changes in the anterior pituitary at 3600 ppm was evident. These lower incidences of pituitary proliferative changes with high-dose sedaxane treatment are an Associative Event in the MOA, as the age-related increase in pituitary adenomas in control rats is known to reflect loss of dopaminergic suppression of the pituitary lactotroph cells (Freeman *et al.*, 2000).

There were no differences from controls in findings in the mammary gland or the pituitary gland at the 1-year sacrifice (data not shown); therefore, the data indicate that these effects were late onset findings, *i.e.,* after 1 year of exposure.

By looking across all treatment groups in the 2-year rat study with sedaxane, the registrant concluded that on an individual animal basis, certain other patterns and correlations are noteworthy:

- Notably, in animals with uterine adenocarcinomas (regardless of dose group), there is no co-incidence of mammary gland fibroadenomas.
- Considering the large deficits in body weight gain vs. control in the 3600 ppm females and (to a lesser extent) in the 1200 ppm females (**Table 45**), along with the patterns of tumor incidence shown in **Table 47**, there is a clear relationship between average weekly body weight gain and the incidences of these tumor types.
    - Mammary gland fibroadenomas and pituitary adenomas occur in rats with higher body weight gains, and uterine adenocarcinomas occur in rats with lower body weight gains. This correlation is illustrated graphically in **Figure 10**.

Figure 10: Relationship of Average Weekly Body Weight Gain with Tumor Type in Sedaxane 104-Week Rat Study



The box extends from the 25th and 75th percentiles of average weekly body weight gain while the whiskers are the minimum and maximum average weekly body weight gain. The line within the box is the median while the plus sign is the mean weekly body weight gain. Data from all female rats in this study, regardless of treatment group, were combined in this analysis. Data from Perry, 2010b (MRID 47473386).

In summary, lower incidences of proliferative responses in the pituitary (adenomas) and mammary gland (fibroadenomas) occur only in the 3600 ppm females, and the registrant stated these data provide strong evidence that prolactin is an integral component of the proposed MOA.

The registrant concluded that these data are consistent with the caloric restriction-mediated changes in tumor profiles in Wistar rats (Harleman *et al.*, 2012; Roe *et al.*, 1995; Tucker, 1979), regarding Biological Plausibility of the proposed MOA.

CARC concluded that sedaxane treatment at 3600 ppm leads to a decrease in mammary gland (statistically significant at p<0.001) proliferative findings in female rats (Associative Events 2 and 3 for the mode of action hypothesis presented in Figure 9). Also in the 3600 ppm sedaxane treated animals, there was a statistically significant decrease (p<0.001) in the incidence of "no abnormality detected" and lobular hyperplasia with atypia in the mammary gland. However, there is no direct evidence provided by the registrant linking the mammary gland observations to decreased prolactin levels. In addition, as discussed in more detail below, there is no direct evidence to support a decrease in serum prolactin levels, as inferred by the registrant.

d1) Suppression of age-related decrease in dopaminergic signaling in the hypothalamus

The following text in Section d1 was taken directly from the Registrants proposed MOA package (MRID 49804813)

As outlined in **Figure 9**, the registrant-proposed MOA includes the preservation of dopaminergic signaling in the TIDA neurons of the hypothalamus as rats age, possibly *via* altered serum levels of leptin, adiponectin, and other adipokine signals to the hypothalamus, which are directly proportional to body weight, nutritional status, and adipose tissue content of the animals (Arner, 2003; Tena-Sempere, 2015; Woodside *et al.*, 1998). To help orient the reviewer of this Assessment to the underlying physiology, **Figure 11** provides a schematic of the TIDA region and how it relays dopamine to the anterior pituitary to control the release of prolactin.

The TIDA neurons have their cell bodies in the arcuate nucleus (ARC) region of the hypothalamus, and their axons extend into the median eminence (ME) region. These TIDA neurons synthesize dopamine, which is released from the ME and travels *via* the primary plexus (a network of capillaries) into the anterior pituitary. In the pituitary, dopamine has an inhibitory effect on the lactotrophs that synthesize and release prolactin into the systemic circulation. Circulating prolactin also plays a role in down-regulating dopamine release by the TIDA neurons, completing a known negative feedback loop.

Figure 11: Anatomy of the TIDA Neurons and Control of Prolactin Release in the Anterior Pituitary

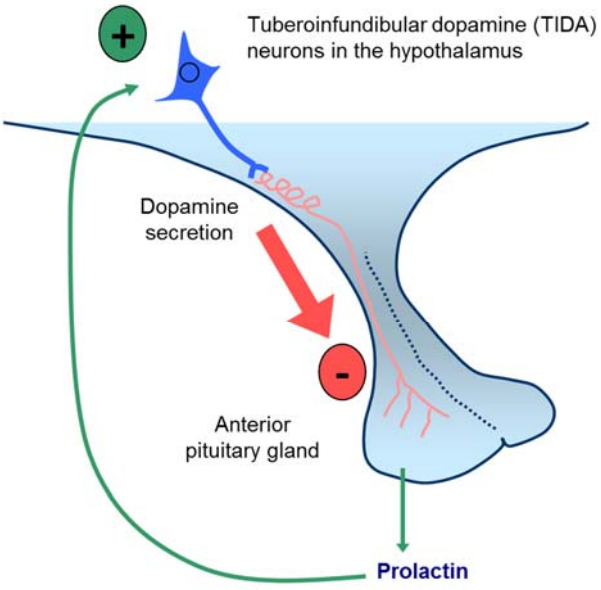


Image provided by Dr. Dave Grattan, Univ. of Otago

d2) Age-related changes in dopaminergic neurons as indicated by tyrosine hydroxylase mRNA and protein expression in isopyrazam treatment groups

Isopyrazam - Evaluation of Hypothalamic Tyrosine Hydroxylase in Control Female Wistar Rats at 3, 12 or 24 months by Immunohistochemistry and *in-situ* Hybridization (MRID 49804815)

Prior published work has outlined a hypothesized mechanism where large reductions in body weight due to caloric restriction can produce a shift in uterine, mammary, and pituitary tumor incidence *vs.* untreated controls, *via* a delay in reproductive senescence of Wistar rats (Ben-Jonathan, 1985; Harleman, *et al*., 2012; Roe, *et al*., 1995). The delayed senescence is postulated to arise *via* a protective effect that prevents the normal age-related decrease in dopaminergic neurons in the TIDA region. Greater secretion of dopamine from the TIDA neurons compared to control rats at 12 - 24 months of age would result in a sustained inhibition of prolactin (PRL) release from the anterior pituitary. This maintenance of dopaminergic inhibition of prolactin secretion would result in prolongation of estrus cycling with resultant continued intermittent estrogenic stimulation of the uterine epithelium, for a longer period of time than normal, and uterine proliferative change.

Sanchez and coworkers (Sanchez, *et al.,* 2003) found that aging rats lose dopaminergic neurons in the hypothalamus *via* a process of senescence. Senescence occurred during normal aging in the TIDA neurons of the hypothalamus, and was reflected by reduced secretion of dopamine into the hypophyseal portal blood vessels, which convey this signal to the anterior pituitary (Gudelsky and Porter, 1981; Reymond and Porter, 1981).

The mRNA and protein expression of tyrosine hydroxylase (TH), a rate limiting enzyme for dopamine synthesis in hypothalamic arcuate nucleus (ARC) and median eminence (ME) neurons of the tuberoinfundibular dopaminergic (TIDA) neurons, was evaluated using immunohistochemistry (IHC) and RNAscope™ *in situ* hybridization (ISH) staining. The TIDA neurons have their cell bodies or perikarya located in the ARC and project their axons into the ME. These neurons lack true synapses, and dopamine secreted by axons of these TIDA neurons is carried *via* the capillaries of the hypophysial portal blood to the anterior pituitary, where it activates the dopamine-2 (D2) receptors of lactotrophs, thus inhibiting the secretion of PRL by the pituitary (Lookingland, 2005).

Immunohistochemistry (IHC) is a standard immunological technique to detect protein expression in FFPE (formalin-fixed, paraffin-embedded) tissue sections. Staining of TH by IHC has been used previously to visualize dopaminergic neurons in rat hypothalamus (Sanchez *et al.* 2003). RNAscope™ *in situ* hybridization (ISH) is capable of visualizing cellular expression of 'low copy number' (<20 mRNA copies per cell) mRNAs in FFPE samples. This method can detect down to a single mRNA molecule per cell (Wang *et al*., 2012) and hence provides the facility to quantify mRNA expression levels in tissues/cells.

In the present study, the effect of aging on tissue sections (FFPE) from the hypothalamus of control female Wistar rats were examined to determine changes over time in the number of dopaminergic (tyrosine hydroxylase (TH)) neurons. The control female rats were obtained from the 90-day (MRID 47746834) and combined 12-month and 24-month dietary studies (MRID 47746851) with isopyrazam.

The results from the 90-day, 12-month and 2-year control groups were compared to look for trends in IHC staining of the TIDA region with age (**Figure 12**). For each age group, the IHC

staining displayed higher amounts in the ME region than in the ARC region of the TIDA, which is consistent with localization of TH protein to a greater extent in the axons (ME) than in the cell bodies (ARC). Image analysis of the TH immunostained ARC alone, ME alone and ARC+ME regions of interest (ROIs) indicated that mean values were statistically significantly lower in all three regions for the 2-year animals compared to the 1-year animals. By contrast there was a slight but significant ($p<0.05$) increase in TH protein staining in the ME and ARC+ME regions of the 1-year control animals compared to the 90-day control animals.

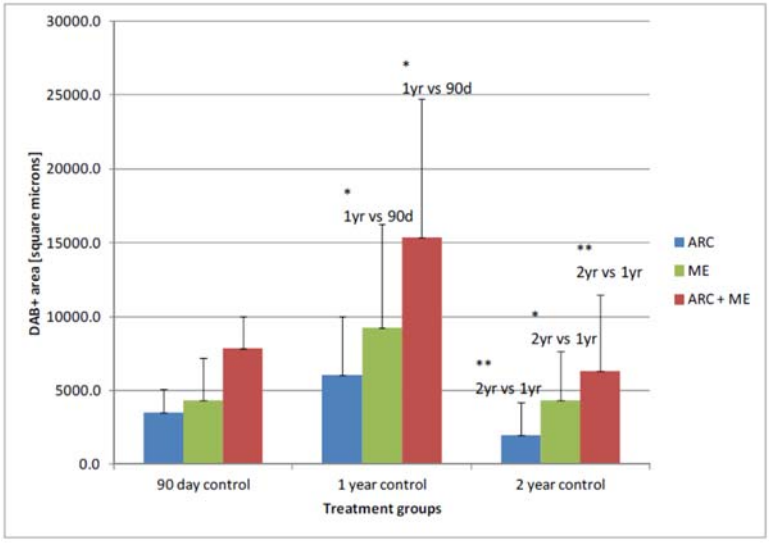


Figure 12: Quantification of TH protein in the TIDA region based on the IHC labeling in control groups of increasing age.

Histogram shows the DAB positive area (μM^2) for IHC staining in the ARC only, ME only and ARC+ME of the TIDA for 90-day, 12-month and 2-year control animals. Results are mean (DAB +ve threshold/ROI area μM^2) ± SD, n=8-10.

* statistically significantly different $p<0.05$, by students t-test 1 tailed type 2

**statistically significantly different $p<0.01$, by students t-test 1 tailed type 2

The results from the 90-day, 12-month, and 2-year control groups were compared to look for trends in ISH staining of the TIDA region with age (**Figure 13**). In all of the age groups, the ISH staining for TH mRNA was predominantly in the ARC, which is consistent with localization of TH mRNA predominantly in the cell bodies (ARC) rather than the axons (ME). Image analysis of the TH ISH-stained mRNA in the ARC alone and ARC+ME ROIs indicated that there was a statistically significantly lower ($p<0.01$) level of TH mRNA staining in these regions for the 2-year animals compared to the 90-day controls. Although the 12-month control TH ISH staining in the ARC alone and ARC+ME regions appeared lower than in the 90-day control group, this was not statistically significant due to large standard deviations for data in these groups. Plots of the individual animal data for ISH staining in the ARC alone and the ARC+ME regions also reflected these same patterns, including the general trend of lower values from 90 days to 1 year to 2 years in control animals. There was a significantly ($p<0.01$) higher level of TH mRNA staining in the ME alone of the 2-year control group compared to the 90-day control group; however, the level of TH mRNA staining in this region was very low compared to the ARC region (>10 fold lower than the ARC), and therefore this apparent difference is not considered biologically significant.

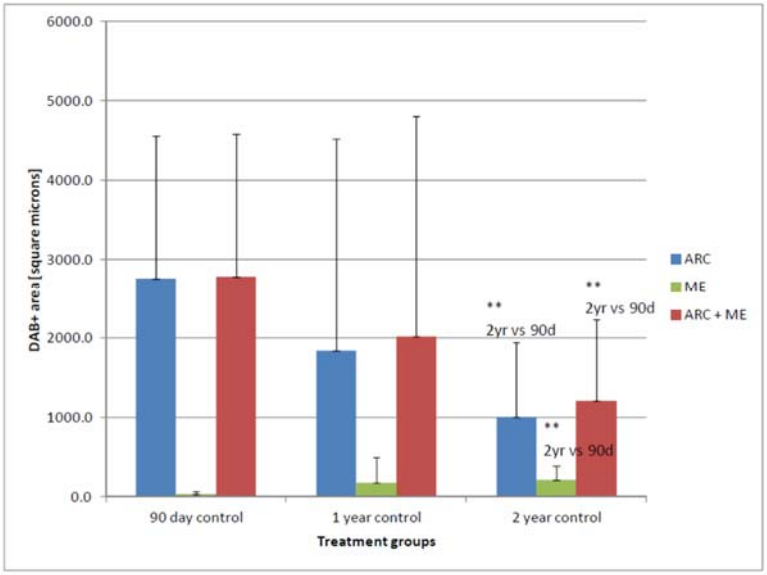


Figure 13: Quantification of TH mRNA in the TIDA region based on the ISH labeling in control groups of increasing age.

Histogram shows the image analysis data for ISH staining in the ARC only, ME only and ARC+ME of the TIDA for 90-day, 12-month and 2-year control animals. Results are mean (DAB +ve threshold/ROI area μM^2) ± SD, n=8-10.

* statistically significantly different $p<0.05$, by students t-test 1 tailed type 2

**statistically significantly different $p<0.01$, by students t-test 1 tailed type 2

CARC concluded that the TH protein measured in the TIDA region was not significantly decreased at 2 years as compared to 90 days. The mRNA staining levels were decreased in one and two-year-old control animals, however, there was no overall correlation in change between mRNA and protein staining (this information will help support key event(s) for the mode of action hypothesis presented in Figure 9).

d3) Changes in dopaminergic neurons as indicated by tyrosine hydroxylase mRNA and protein expression in sedaxane treatment groups (Key Events 3, 4 & 5)

Sedaxane- Analysis of Stored Tissue from 2-Year Rat Study for Hypothalamic Tyrosine Hydroxylase *via* Immunohistochemistry and *in situ* Hybridization (MRID 49804816)

Sedaxane was evaluated for effects on the expression level of TH in animals from the 2-year chronic carcinogenicity study (control, 1200ppm and 3600ppm) (MRID 47473386). Hypothalamus sections from 2-year control (30 samples), 1200 ppm (30 samples) and 3600 ppm (27 samples) animals were immunostained for TH IHC. From these stained slides, 36 samples in total (twelve control, twelve of the 1200 ppm animals and twelve of the 3600 ppm animals) were selected for further image analysis. For each treatment group, the IHC staining displayed higher amounts in the ME region than in the ARC region of the TIDA, which is consistent with localization of TH protein to a greater extent in the axons (ME) than in the cell bodies (ARC). The IHC image analysis data are shown in **Figure 14**. There was a statistically significant ($p<0.01$ or $p<0.05$) higher TH protein level in the ARC only, ME only, and combined ARC+ME regions in the 1200 ppm group compared to control. There was also a statistically significant

effect ($p<0.01$ or $p<0.05$) on TH protein level in the ME only and ARC+ME regions in the 3600 ppm group compared to control.

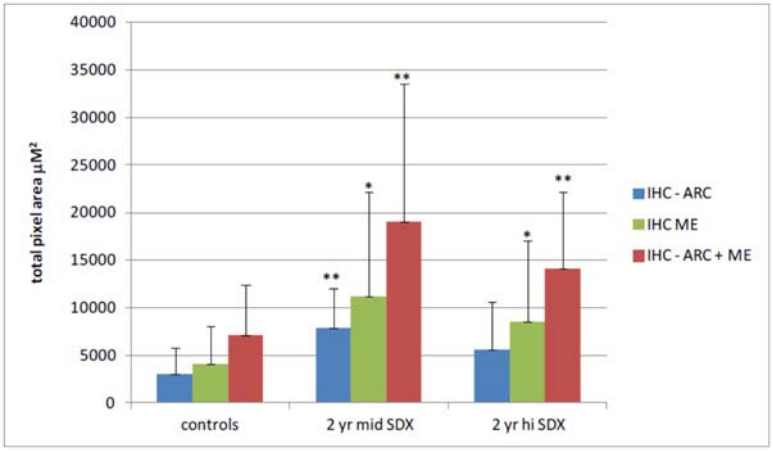


Figure 14: Quantification of TH protein in the TIDA region based on the IHC labeling.
Histogram shows the DAB positive area (μM^2) for IHC staining in the ARC only, ME only and ARC+ME of the TIDA for control, mid-dose (1200 ppm) and high-dose (3600 ppm) SDX treated animals. Results are mean (DAB +ve threshold/ROI area μM^2) ± SD, n=12.
* statistically significantly different from control at $p<0.05$, by students t-test 1 tailed type 2
**statistically significantly different from control at $p<0.01$, by students t-test 1 tailed type 2

Hypothalamus sections from 2-year control (30 samples), 1200 ppm (12 samples), and 3600 ppm (27 samples) animals were stained for ISH of TH. From these stained slides, 36 samples in total (twelve control, twelve of the 1200 ppm animals and twelve of the 3600 ppm animals) were selected for further image analysis. The animals selected for TH ISH image analysis were the same as those selected for the TH IHC image analysis and this was performed in the same way on both samples. In all of the treatment groups, the ISH staining for tyrosine hydroxylase mRNA was predominantly in the ARC, which is consistent with localization of TH mRNA predominantly in the cell bodies (ARC) rather than the axons (ME). The ISH image analysis data are shown in **Figure 15**. There was a statistically significant ($p<0.05$) higher TH RNA expression in the ARC and ARC+ME regions in the 3600 ppm group compared to the controls. In contrast, there were no statistically significant differences in TH mRNA expression in the 1200 ppm treated group compared to the controls.

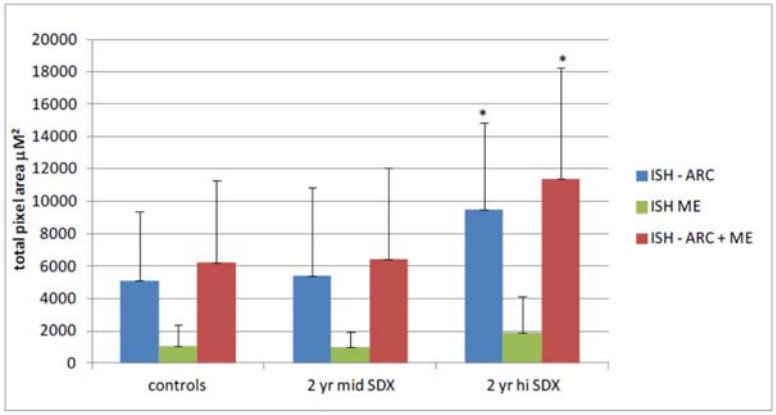


Figure 15: Quantification of TH mRNA in the TIDA region based on the ISH labeling.

Histogram shows the ISH staining in the ARC only, ME only and ARC+ME of the TIDA for control, mid-dose (1200 ppm) and high-dose (3600 ppm) SDX treated animals. Results are mean (DAB +ve threshold/ROI area μM^2) ± SD, n=12.
* statistically significantly different from control at $p<0.05$, by students t-test 1 tailed type 2

CARC concluded that TH protein levels were statistically significant higher in the 1200 ppm and 3600 ppm sedaxane-treated groups as compared to the control. However, the 1200 ppm group had higher TH protein levels as compared to the 3600 ppm sedaxane-treated group. The TH mRNA were statistically significant higher at 3600 ppm as compared to the controls.

An additional study (MRID 50101901) was submitted after the registrant submitted the proposed MOA (MRID 49804813). A short review of this study related to serum prolactin, leptin, and adiponectin levels is presented below.

In rats terminated at the 1-year (52-week) sacrifice, no apparent differences in prolactin levels were discernible in control and sedaxane-treated groups. A high level of variation between individual animals in prolactin levels at 52 weeks of age was expected based on cycle state, timing of reproductive senescence as well other factors (e.g. presence of pituitary adenomas) in Wistar rats of this age (Mitchard and Klein, 2016; Lu et al., 1979). This large variability as well as small sample sizes for animals in specific estrous cycle stages made the detection of any statistically significant differences between control and sedaxane-treated groups for prolactin levels extremely unlikely from the available serum samples in this study.

Because leptin and adiponectin are not known to vary based on reproductive cycle state, these values were less variable than prolactin levels after 52 weeks. There were no statistically significant differences in adiponectin or leptin levels between control and treatment groups, although there was a trend for decreased leptin in the 3600 ppm group, which was correlated to the 13% lower body weight value in this same treatment group. Leptin, produced by adipocytes, is known to be directly proportional to percent body fat in rats (Tena-Sempere, 2015; Wolden-Hanson *et al*., 1999), and thus to body weight. The observed difference between the 0 and 3600 ppm groups is highly plausible based on the known biology of leptin, and the 18% lower mean value for leptin in 3600 ppm females at 52 weeks is considered to be a treatment-related effect according to the registrant.

The CARC concluded that the suppression of age-related increase in prolactin was not supported by serum prolactin data. There is also no direct evidence supporting a sedaxane-induced correlation between dopaminergic and prolactin levels. The data provided on leptin and adiponectin levels do not provide additional significant evidence to support any of the Key Events in the registrant proposed MOA.

e1) Reproductive senescence in sedaxane-treated female rats (Associative Event 4 & Key Event 6)

The following text in Section e1 was taken directly from the Registrants proposed MOA package (MRID 49804813).

In female Wistar rats, most animals are still cycling and reproductively competent at 11-12 months of age. By 17-18 months of age, they move into senescent stages that are dominated by "constant diestrus" [terminology used by Kachi *et al.* (2006); this is more commonly termed "repetitive pseudopregnancy" (vom Saal and Finch, 1988)]. As the animals age (≥24 months), a greater percentage of Wistar rats enter a stage of "persistent anestrus," in which they are acyclic and ovarian atrophy becomes more predominant. The incidence of rats in "persistent estrus" is quite small in aging Wistar rats compared to Sprague-Dawley rats (Eldridge *et al.*, 1999; Wetzel *et al.*, 1994). Similar to the Fischer 344 rat, Wistar rats have a relatively low spontaneous incidence of mammary adenomas and carcinomas compared to Sprague-Dawley rats, which is consistent with a change to repetitive pseudopregnancy where the estradiol:progesterone ratio would remain relatively low compared to aging Sprague-Dawley rats, which experience longer periods of higher endogenous estrogen exposure.

Several lines of evidence are available regarding the effects of sedaxane on female Wistar rats related to aging and reproductive senescence. First, the original pathology data from the 2-year rat study (Perry, 2010b) (MRID 47473386) showed a significantly lower incidence of vaginal mucification in the 3600 ppm treated females, as shown in **Table 49**.

The pathology data from the 2-year rat study showed a lack of difference between control and treated groups after 1 year for findings in the vagina. All animals appeared to be in some stage of estrous cycling, and a very low incidence of vaginal mucification (0 – 2) was observed in any group. Mucification of the vagina is a trait that commonly occurs in repetitive pseudopregnancy (Westwood, 2008). It can also be observed to a lesser degree in other stages of the estrous cycle, particularly during irregular cycles prior to progression into reproductive senescence. This is consistent with previous observations that most Wistar rats of this age experience normal estrous cycles.

In the original study report, a statistically higher number of rats in the 3600 ppm sedaxane group were observed with "no abnormality detected" in the vagina (**Table 49**). According to the registrant, the major contributor to this difference was mucification of the vagina, which was significantly lower than controls in the 3600 ppm group. Based on these observations, the pathology data suggested that a lower number of rats in the 3600 ppm group had progressed into repetitive pseudopregnancy, which in turn suggests that the females in the 3600 ppm group continued to experience estrous cycling for longer than the controls, or enter reproductive senescence later in life, as concluded by the registrant.

TABLE 49: Incidence of Pathology Findings in the Vagina at 1 Year and 2 Years in the Carcinogenicity Study (Female Rats) as a Marker of Reproductive Senescence

Histopathology findings	2-year carcinogenicity study			
	0 ppm	200 ppm (14 mg/kg/day)	1200 ppm (86 mg/kg/day)	3600 ppm (261 mg/kg/day)
Vagina – 1 Year (sacrifice)	(12)	(12)	(12)	(12)
No abnormality detected	12	10	11	10
Mucification	0	2	1	2

Histopathology findings	2-year carcinogenicity study			
	0 ppm	200 ppm (14 mg/kg/day)	1200 ppm (86 mg/kg/day)	3600 ppm (261 mg/kg/day)
Estrous cycle: diestrus	5	5	8	4
Estrous cycle: metestrus	3	4	2	3
Estrous cycle: estrus	3	1	0	4
Estrous cycle: proestrus	1	2	2	1
Vagina – 2-Years (survivors + decedents)	(52)	(52)	(52)	(52)
No abnormality detected	23	16	21	35*
Mucification	15	22	16	3**
Atrophy	13	11	14	11
Inflammation / inflammatory cell infiltration	0	5	0	0
Squamous cell hyperplasia +/- inflammation	0	0	0	1

Data from Perry, (2010b) (MRID 47473386), are pathology incidences in the original report from Charles River Laboratories, UK.
*, ** statistically significant by pairwise Fisher's Exact Test ($p<0.05$, 0.01)

Microscopic Evaluation of Vagina, Uterus, and Ovary from Subchronic and Chronic Rat Dietary Studies to Determine Cycle Stage (MRID 49804814)

To further investigate the possible age-related changes in organs related to estrous cycling, histology of the vagina, ovaries, and uterus from existing histology slides from a 90-day study in Wistar rats (Shearer, 2009) (MRID 47473376) and the 2-year chronic/carcinogenicity study in Wistar rats (Perry, 2010b) (MRID 47473386) were re-evaluated by an independent pathologist (MRID 49804814). The objective of this work was to determine the cycle state from the microscopic examination of the vagina, uterus, and ovary of female rats exposed to sedaxane in their diets for intervals ranging from 13 to 104 weeks. Slides from each rat were coded and divided into the following Subgroups (A – E) based on time of death or sacrifice, as follows:

Subgroup	Time Period/ Fate	Total Number of Rats
A	13 weeks – Scheduled sacrifice	20
B	0-52 weeks – Animals deceased	5
C	52 weeks – Scheduled sacrifice	33
D	53-104 weeks – Animals deceased	29
E	104 weeks – Scheduled sacrifice	125

The pathologist was aware of the subgroups for each animal but blind to the treatment groups. After recording relevant findings in each of the three tissues (where possible), an overall estimate of the cycle stage (*e.g.,* metestrus, diestrus, proestrus, or estrus for cycling rats) or the senescent stage (*e.g.,* repetitive pseudopregnancy, persistent estrus, or persistent anestrus) was recorded under "vagina" for that animal. In addition, for cycling rats, if the ovary and uterus had changes that did not match the agreed cycle stage from the vagina, an additional descriptor of "asynchronous" was recorded. The histology as defined by Westwood (2008) in vagina, ovaries, and uterus was used as a guide to facilitate making a determination of cycle stage or senescent stage for each animal. In addition, specific histology descriptors related to cycle stage were recorded for each tissue (vagina, ovaries or uterus) based on a pre-determined glossary that was defined in the study protocol. The results were peer-reviewed before unblinding the study. The

incidences of each stage/finding by treatment group were tabulated. A summary of findings for rats that lived beyond 52 weeks (*i.e.,* Subgroups D+E combined) is shown in **Table 50**.

TABLE 50: Summary Findings – Histology Re-evaluations of Control and Sedaxane-Treated Tissues Related to Cycle Stage: Combined 104-Week Sacrifice + Decedents from Weeks 53-104.

Time Groups D + E	Combined Incidence		
	0 ppm	1200 ppm (86 mg/kg/day)	3600 ppm (261 mg/kg/day)
Vagina Descriptive Findings (N)	(50)	(52)	(51)
Nothing Abnormal Discovered	4	4	1
Epithelium; Inactive	15	18	27
Mucification	29	29	21
Cornification	2	1	2
Vagina Estrous Cycle (N)	(50)	(52)	(51)
Repetitive Pseudopregnancy	29	28	19
Persistent Anestrus	15	18	27
Persistent Estrus	0	1	1
Total – Senescent Stages	44	47	47
Diestrus	2	2	1
Metestrus	1	2	0
Estrus	2	0	1
Proestrus	1	1	2
Total – Cycling Stages	6	5	4

For additional descriptive findings and results for other time intervals, see original report (MRID 49804814)

The registrant concluded that the results indicate that in younger animals (*i.e.,* from 13 weeks up through the 52-week sacrifice), the three tissues showed correlation with respect to cyclicity state, and the majority of rats in all groups were experiencing estrous cycles (data not shown – incidences consistent with prior results in **Table 49**).

In contrast, a lack of concordance across these three tissues was the norm in animals from 53 – 104 weeks of age. The study pathologist indicate that the criteria described by Westwood (2008) for vagina, uterus, and ovaries in the senescent stages are idealized because as the animals age with respect to reproductive senescence, there is a great deal of variability across the three tissues, as well as within individual tissues. Therefore, a lack of concordance across the three tissues is not unexpected in senescent rats.

In the re-evaluation, there was a marginally lower incidence of vaginal mucification and the corresponding cycle stage of "repetitive pseudopregnancy" in the 3600 ppm animals at 53-104 weeks (**Table 50**). The total incidences of vaginal mucification in the control, 1200 and 3600 ppm groups were numerically higher in each group than those in the original pathology read (**Table 49**), which according to the registrant is an expected outcome as this histology re-evaluation was designed to focus on subtle changes related to reproductive cycle stages in these tissues. In contrast, the original pathology evaluation was for the purpose of finding adverse pathological changes including neoplasias. In summary, the registrant concluded that the blinded

histology re-evaluation confirmed the original study result of a lower incidence of vaginal mucification, and further confirmed that this translated to a lower incidence of repetitive pseudopregnancy in the 3600 ppm females at 53-104 weeks.

Because there were very few rats that died between 53 and 104 weeks (Subgroup D) [see study report (MRID 49804814)], clear conclusions cannot be made for this age grouping. The majority of animals in **Table 50** were from the 104-week sacrifice. There was a higher incidence of aging animals in the 3600 ppm group that were considered to be in persistent anestrus, based on vaginal findings plus confirmatory changes in ovaries and uteri. In contrast, there were no differences from the control group in any histology findings for the 1200 ppm females (**Table 50**).

The registrant concluded that the results of this histology re-evaluation indicated that the predominant senescent stages in older Wistar rats were repetitive pseudopregnancy or persistent anestrus. Moreover, incidences of persistent estrus were minimal, confirming the tendency of Wistar rats to enter senescent stages of repetitive pseudopregnancy (*i.e.,* senescent mucification) and eventually, persistent anestrus (Kachi *et al.*, 2006).

The blinded re-evaluation of the three tissues showed no clear differences in cycle stage or overall senescence for female rats treated at 3600 ppm sedaxane *vs.* controls at 53-104 weeks; further, it confirmed a lack of any differences from controls at 1200 ppm, as concluded by the registrant. However, the mucification data do not rule out that a difference with treatment may have occurred at some time during the 53 – 104-week period when Wistar rats enter senescence. Examination of a limited set of tissue sections at a single point in time cannot provide sufficient details to fully understand the continuum of changes that occurred during reproductive senescence in these groups of female rats.

The registrant concluded that these data are consistent with the caloric restriction-mediated changes in reproductive senescence in Wistar rats, as discussed in Section XVI. 1a regarding Biological Plausibility of the proposed MOA. As proposed (**Figure 9**), most Wistar rats begin to cycle irregularly and enter various senescent stages somewhere between 53 and 80 weeks of age (Kachi *et al.*, 2006). Prior long-term studies in rats have demonstrated that some individuals may experience irregular cycles, followed by repetitive pseudopregnancy and persistent anestrus, while others may proceed from irregular cycles directly to persistent anestrus (vom Saal and Finch, 1988). Particularly in the presence of a massive body weight deficit and associated neuroendocrine changes that have been suggested at 3600 ppm, it is reasonable to conclude, according to the registrant, that high-dose sedaxane treatment altered the time course and the nature of these time-dependent changes in cycle stage. Thus, a higher incidence of 3600 ppm rats in persistent anestrus and a lower incidence of rats in repetitive pseudopregnancy is a plausible outcome of a difference in the transition into senescence over time in this treatment group.

CARC concluded that there was a modestly lower incidence of vagina mucification in the high-dose group (3600 ppm) with no clear differences in cyclicity or overall senescence based on the histopathology re-evaluation. Measurements were taken when all groups had high rates

of senescence (Associative Event 4 and Key Event 6 for the mode of action hypothesis presented in Figure 9). Moreover, the CARC concluded that there was no evidence of squamous metaplasia, endometrial hyperplasia, or other proliferative lesions indicative of prolonged estrogenic stimulation at one or two years.

e2) Sedaxane - 13 Week Rat Dietary Toxicity Study (MRID 47473376)

The following text in Section e2 was taken directly from the Registrants proposed MOA package (MRID 49804813).

In a subchronic 90-day rat study, a limited number of parameters associated with the proposed MOA (vagina, ovary, and uterus pathology and ovarian weights) were assessed (Shearer, 2009) (MRID 47473376). These data for the female rats are summarized in **Table 51**. There were no effects of sedaxane treatment on ovary weights, uterine weights and pathology findings in the ovaries, uterus, and vagina. Cycle status was evaluated based on the histology of the vagina, and all rats appeared to be cycling similarly in the control and 3600 ppm groups. Metestrus and diestrus are overlapping portions of the cycle in rats that are sometimes referred to as diestrus 1 and diestrus 2, and the total incidence of rats in a combination of these two stages was the same in the control group (6) as in the high dose group (6).

TABLE 51: Summary of Data from a 13-Week Rat Study with Sedaxane –Parameters Potentially Related to the Uterus MOA

	0 ppm	300 ppm (28 mg/kg/day)	2000 ppm (186 mg/kg/day)	4000 ppm (350 mg/kg/day)
Organ Weights:				
Terminal body wt. (g)	243	239	221*	214**
Ovary wt. (g)	0.104	0.106	0.102	0.095
Ovary wt. adjusted for body weight (g)	0.097	0.100	0.106	0.104
Uterus wt. (g)	0.59	0.71	0.59	0.56
Uterus wt. adjusted for body weight (g)	0.61	0.72	0.58	0.54
Histopathology: (N)	(10)	(0)	(0)	(10)
Ovary:				
No abnormality detected	10	-[a]	-	10
Uterus:				
No abnormality detected	10	-	-	10
Estrus dilation	0	-	-	1
Vagina:				
No abnormality detected	10	-	-	10

	0 ppm	300 ppm (28 mg/kg/day)	2000 ppm (186 mg/kg/day)	4000 ppm (350 mg/kg/day)
Estrous cycle: diestrus	0	-	-	3
Estrous cycle: metestrus	6	-	-	3
Estrous cycle: estrus	4	-	-	4

*, ** Statistically-significantly different from control with $p<0.05$ and $p<0.01$, respectively.
Data from Shearer (2009) (MRID 47473376).
a "– "indicates no animals at this dose levels were examined for that parameter

Two other subchronic studies in rats (4-week and 13-week studies) were performed earlier, but in a different strain and supplier of rats than the 2-year study (HsdRccHan:WIST, from Harlan Laboratories). These studies were also conducted in a different laboratory (Syngenta Central Toxicology Laboratory, UK) than the 2-year rat study with sedaxane. No relevant findings in the tissues that are pertinent to the proposed MOA for uterine tumors were observed in these studies; therefore, they are not summarized further (Noakes, 2007; Peffer and Noakes, 2010).

CARC concluded that there was no evidence of cyclicity disruption or other reproductive tract abnormalities at 90 days.

XII. APPLICATION OF INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY (IPCS)/INTERNATIONAL LIFE SCIENCE INSTITUTE (ILSI) FRAMEWORK FOR THE EVALUATION OF THE HUMAN HEALTH RELEVANCE OF A HYPOTHESIZED MODE OF ACTION FOR UTERINE TUMORS

A. Postulated MOA and Key Events:

The following narrative were extracted from the registrant submitted MOA and human relevance framework document for rat uterine tumors (MRID 49804813).

Based on the available information, the registrant's representatives postulated that the MOA for sedaxane-induced rat uterine tumors is:

Key events for this MOA include the following:
Decrease in body weight gain
Decrease in adipose tissue
Suppression of age-related decrease in dopaminergic signaling
Suppression of age-related increase in prolactin
Increased age at reproductive senescence
Increase in total number of estrus cycles and proliferation
Increase in uterine adenocarcinomas

Associative events for this MOA include the following:
Decreased signaling to the hypothalamus

Decreased pituitary gland proliferative findings
Decreased mammary gland proliferative findings
Decreased senescent mucification of the vagina

B. Dose-concordance of key events

With respect to the incidence of uterine adenomas and adenocarcinomas in female Han Wistar rats (**Table 44**), dose levels of 200 and 1200 ppm sedaxane (14 and 86 mg/kg/day, respectively) can be considered non-tumorigenic doses. At 3600 ppm (261 mg/kg/day), the tumorigenic dose, the combined incidence of uterine adenomas and adenocarcinomas was statistically significantly higher than concurrent controls, but within the range of historic control values from the test laboratory and the RITA database, especially in light of the rarity of zero incidences of these tumors in the historical control databases. **Table 52** describes the key events observed at each dose level as proposed by the registrant.

The registrant concluded that there is good dose concordance of the proposed key events. The initial key event of decreased body weight was observed throughout the 2-year rat study at 3600 ppm and in a 13-week rat study at a similar high dose level (4000 ppm). As observed in **Table 45**, the divergence of the body weight and body weight gain was accelerated in the second year of the study for the 3600 ppm females compared to the control group. The final body weight gains at this dose relative to the control group was lower by approximately 50%, while in the 1200 ppm group it was approximately 11% lower. Although the percentage of body fat and serum levels of the hormones such as leptin and adiponectin were not specifically measured in the long-term sedaxane rat study, decreases in these parameters secondary to large body weight gain deficits can be inferred from existing literature, as concluded by the registrant. Leptin and adiponectin are two factors that provide feedback signaling to the neuroendocrine control of appetite, energy usage and reproductive control centers in the brain (Tena-Sempere, 2015), and caloric restriction has been consistently shown to be preserve neuronal function in aging rodents (Lin *et al.*, 2015; Pani, 2015). The registrant concluded that consistent with this feedback due to lower body weight gains and adipose tissue, the TIDA neurons in the hypothalamus retain function as indicated by higher levels of tyrosine hydroxylase in the 2 year 3600 ppm group compared to concurrent controls. Specifically, higher levels of TH mRNA and protein were observed only in the 3600 ppm sedaxane group (**Figure 14** and **Figure 15**).

The registrant concluded that associative events present in the histology findings from the 2-year rat study served as markers for two additional proposed key events (**Table 48**):

- In the pituitary, retention of a greater number of functional TIDA neurons in the 3600 ppm sedaxane group resulted in continued production of dopamine, which tonically inhibits prolactin release from the anterior pituitary. This Associative Event is indicated by a lower incidence of proliferative changes in the anterior pituitary and a significantly higher number of animals with no abnormalities in the pituitary. These decreased incidences of proliferative changes as well as pituitary adenomas were observed at 3600 ppm, but not at 200 or 1200 ppm sedaxane treatment.

- In the mammary gland, the resulting lower circulating prolactin levels (due to continued dopamine –mediated tonic inhibition of prolactin release by the pituitary) led to a complete absence of fibroadenomas (0/52) in the 3600 ppm treatment group; in contrast, incidences in the 200 and 1200 ppm groups were similar to control incidences.

TABLE 52: Summary of Dose-Concordance of Associative Events and (Causal) Key Events

Dietary inclusion level of Sedaxane (ppm) [a]	Decrease in body weight gain (Key Event)	Decreased adipose tissue after 1-2 yrs. = Decrease in signals to hypothalamus (*e.g.,* leptin, adiponectin, other factors) (Key Event)	Hypothalamus: Increased DA activity in TIDA neurons after 2 years; ↑ TH mRNA levels [c] (Key Event)	Hypothalamus: Increased DA activity in TIDA neurons after 2 years; ↑ TH Protein levels [c] (Key Event)	Marker of ↑ Dopamine from TIDA: Decreased proliferation in anterior pituitary (Associative)	Marker of ↓ blood Prolactin levels: Decreased mammary gland hyperplasia and fibro-adenoma (Associative)	Decreased senescent mucification of the vagina, plus related changes observed at 2 years (Associative)	↑Age at Reproductive Senescence = ↑ Total number of estrus cycles + uterine endometrial proliferation (Key Event)	Increased Incidence of Uterus Adeno-carcinomas (EPA) (Outcome)
200	No	No	No data	No data	No	No	No	No [d]	No
1200 (2000) [a]	Yes (slight)	Inferred [b]	No	Yes	No	No	No	No [d]	No
3600 (4000) [a]	Yes (large)	Inferred [b]	Yes	Yes	Yes	Yes	Yes	Yes [d]	Yes

200 ppm = 14 mg/kg/day; 1200 ppm = 86 mg/kg/day, 2000 ppm = 186 mg/kg/day, 3600 ppm = 261 mg/kg/day, 4000 ppm = 350 mg/kg/day

[a] Values in parentheses are subchronic dose levels (that are similar to the chronic dose level).

[b] Not specifically measured for sedaxane. Increases in body fat content in aging rats fed *ad libitum* has been well established, and lower percent of body weight as adipose tissue occurs in rats that experience decreases in body weight due to diet restriction. In addition, endocrine signals from adipose tissue to the hypothalamus (*e.g.,* leptin, adiponectin, other factors) are proportional to fat content, and thus lower signaling can be inferred for 3600 ppm (261 mg/kg/day) sedaxane-treated rats.

[c] Tyrosine hydroxylase mRNA and protein = ↑ dopaminergic (DA) neurons in TIDA region = ↑ dopamine release.

[d] Captured from previously column, which is a Marker for these Key Events that occur progressively from 1-2 years.

The registrant concluded that several analyses provided evidence for a change in the transition of aging Wistar rats into reproductive senescence at the 3600 ppm sedaxane dose level. Both the initial study report (**Table 49**) and a blinded, retrospective evaluation of the vagina, ovaries and uterus of rats in the 2-year study (**Table 50**) suggest normal estrous cycling in control and treated Wistar rats at 52 weeks. The majority of control rats transitioned to a state of either repetitive pseudopregnancy or persistent anestrus between 53 and 105 weeks (MRID 49804814). This histology re-evaluation also confirmed that unlike Sprague-Dawley, very few Wistar rats enter persistent estrus as a reproductive senescent stage. For the 2-year time interval, the original pathology results (**Table 49**) and the histology re-evaluation (**Table 50**) observed a lower incidence of senescent mucification (repetitive pseudopregnancy) at the high dose of 3600 ppm. In contrast, there were no effects on any of these markers at 200 or 1200 ppm in the original pathology report (**Table 49**) or in the retrospective histology evaluation (**Table 50**).

As suggested by the registrant, by changing/delaying the transition of 3600 ppm sedaxane-treated rats into reproductive senescence, it can be inferred that these animals were exposed to unopposed estradiol for a greater cumulative period (*i.e.,* a higher estrogen: progesterone ratio for more of their lifetime compared to controls). These key events could not be measured in the available samples from sedaxane studies, but they can be inferred based on the Associative Events suggested in 3600 ppm group (**Table 52**). For the Han Wistar rat, most control Wistar rats enter reproductive senescence *via* repetitive pseudopregnancy (with minimal to no persistent estrus), and the histology re-evaluation of samples from the 2-year sedaxane study confirmed this pattern (MRID 49804814).

Thus, the key events of prolonged exposure of the uterus to unopposed estrogen and increased proliferation of the uterine endometrium occurred at 3600 ppm, as concluded by the registrant, based on the associative events that are markers of these changes (**Table 52**). The final outcome in this MOA, an increased incidence of uterine endometrial adenocarcinomas, was only higher at 3600 ppm as compared to controls.

The registrant concluded that all of the key events and/or the associative events that served as markers for the key events were observed at the tumorigenic dose level of 3600 ppm. At 1200 ppm, a slight effect on body weight gain was observed (-11%), but this was insufficient to produce most of the downstream key events. At 200 ppm, the overall NOAEL in the 2-year rat study, none of the key events occurred.

C. Temporal-concordance of key events

The registrant stated that when the tumorigenic and non-tumorigenic dose levels are considered, the observed effects on parameters associated with the key events occur in a logical, time-dependent manner consistent with the proposed MOA. The temporal-concordance is summarized in **Table 53**.

The observed steps occur in a logical sequence, with the key events preceding the time of occurrence of uterine adenocarcinomas. In particular:

- A decrease in body weight gain was observed in the first week of treatment at 3600 ppm, and continued to progress for the duration of the 2-year study. As shown in **Table 45**, the divergence of body weight in the 3600 ppm group from the control group was accelerated in the second year of the study.
- Considering the large deficit in body weight gain at 52 weeks (-34%) and 105 weeks (-50%), it can be inferred that rats in the 3600 ppm group had lower percentages of adipose tissue and thus lower percentages of circulating factors such as leptin and adiponectin.
- Retention of dopamine release from the TIDA region (*i.e.,* decreased pituitary proliferation) and the resulting lower circulating prolactin levels (*i.e.,* decreased mammary gland hyperplasia and fibroadenoma) were clearly suggested in the 3600 ppm group compared to controls at the 104-week sacrifice (**Table 47**). For the low numbers of animals that died between 53-104 weeks, there is some numerical and statistically significant data that suggests these markers were also affected at 3600 ppm sedaxane between 53 and 104 weeks (*e.g.,* a statistically higher incidence of "no abnormality detected" in the mammary glands).
- There was a decreased in incidence of vaginal mucification in in the 3600 ppm rats at 104 weeks (**Table 49**), and this treatment-related difference (representing fewer animals in repetitive pseudopregnancy) was confirmed in a retrospective histology re-evaluation of the vagina, ovaries and uterus (**Table 50**).
- A higher incidence of uterine adenocarcinomas was observed after 104 weeks. Of the 14 animals with this tumor type, only 4 were observed in late decedent animals (after week 89). Therefore, uterine adenocarcinomas appeared after 1.5 – 2 years.
- Notably, clear data on certain time-sensitive key events are available from groups of rats that were sacrificed after 13 weeks of treatment at dose levels up to 4000 ppm sedaxane (**Table 52**) and after 52 weeks of treatment (**Table 49** and **Table 50**). At both time intervals, the majority of rats were cycling. Only 0 or 1 rat per treatment group may have started to show evidence of repetitive pseudopregnancy after 52 weeks [MRID 49804814]. In addition, there were no histopathology changes related to treatment in the vagina, ovaries or uterus, and no effects on relevant organ weights (*e.g.,* ovaries or uterus) at these time points as indicated in the original chronic report. Therefore, the lack of effects on these endpoints is consistent with a mode of action where:
    - Large deficits in body weight gain, altered cycle stage progression and/or effects of treatment on the transition into reproductive senescence occur after 53 weeks, and
    - Any histological changes in the reproductive tissues (vagina, ovaries, uterus) related to the MOA only occur between 53 and 104 weeks of age.

The registrant concluded that the time course of key and associative events, as well as other supporting data, are consistent with the proposed MOA whereby measurable changes on the target tissue (uterus) only begin to appear between 53-104 weeks. Conversely, the lack of any earlier effects on the uterus or other endocrine-sensitive tissues precludes alternative MOAs involving short-term, direct effects on these systems by sedaxane.

TABLE 53: Summary of Temporal Concordance of Associative and (Causal) Key Events

Dietary inclusion level of Sedaxane (ppm) [a]	Decrease in body weight gain (Key Event)	Decreased adipose tissue after 1-2 yrs. = Decrease in signals to hypothalamus (*e.g.,* leptin, adiponectin, other factors) (Key Event)	Hypothalamus: Increased DA activity in TIDA neurons after 2 years; ↑ TH mRNA and Protein levels (Key Event)	Marker of ↑ Dopamine from TIDA: Decreased proliferation in anterior pituitary (Associative)	Marker of ↓ blood Prolactin levels: Decreased mammary gland hyperplasia and fibro-adenoma (Associative)	Decreased senescent mucification of the vagina, plus related changes observed at 2 years (Associative)	↑Age at Reproductive Senescence = ↑ Total number of estrus cycles + uterine endometrial proliferation (Key Event)	Increased Incidence of Uterus Adeno-carcinomas (EPA) (Outcome)
1 – 13 weeks	Yes	Possibly[a]	No data	No data	No	No	No	No
13 – 52 weeks	Yes	Likely[a]	No data	No data	No	No	No	No
53 weeks	Yes	Likely[a]	No data	No data	No	No	No	No
53 – 104 weeks	Yes	Likely[a]	No data	Possibly[c]	Possibly[c]	Likely[b]	Likely[b]	No
104 weeks	Yes	Likely[a]	Yes	Yes	Yes	Yes	Yes	Yes

[a]Not specifically measured for sedaxane. Increases in body fat content in aging rats fed *ad libitum* has been well established, and lower percent of body weight as adipose tissue occurs in rats that experience decreases in body weight due to diet restriction. In addition, endocrine signals from adipose tissue to the hypothalamus (*e.g.,* leptin, adiponectin, other factors) are proportional to fat content, and thus lower signaling can be inferred for 3600 ppm (261 mg/kg/day) sedaxane-treated rats based on the significantly lower body weights.

[b]Histology re-evaluation of vagina, ovaries and uterus from rats that died from 53 – 104 weeks (MRID 49804814) provided insufficient power to assess whether a difference in senescent stages was apparent in this age range. However, changes in 3600 ppm (261 mg/kg/day) females at this time interval are considered highly likely based on clear differences at 104 weeks (**Table 52** and **Table 53**) and the known biology of reproductive senescence in Wistar rats.

[c]See **Table 51** – incidences in decedent animals

D. Strength, consistency, and specificity of the key events and tumor response

As recommended in an IPCS Framework analysis (Boobis *et al.*, 2006), this section discusses the weight of evidence linking the key events, precursor lesions, and the tumor response as suggested by the registrant. Consistent observations in a number of such studies with differing experimental designs increase support for a proposed MOA, since different designs may reduce unknown biases or confounding factors.

For sedaxane, there was no evidence for a treatment-related effect on the incidence of uterine tumors in an 80-week study in CD-1 mice (MRID 47473388). In this study, male and female CD-1 mice were treated with sedaxane at 25, 157 and 900 mg/kg/day for males, and 29, 185 and 1001 mg/kg/day for females, corresponding to dietary inclusion levels of 200, 1250 and 7000 ppm respectively for both sexes. A lack of uterine tumors in mice is consistent with the lack of an excessive effect of sedaxane on body weight, as suggested by the registrant, as the 7000 ppm group only experienced ~9% decrease in body weight compared to controls after 80 weeks of treatment. In addition, the transition of CD-1 mice into reproductive senescence could differ from that in Wistar rats. A lack of uterine tumors in mice following lifetime exposure to a limit dose of sedaxane (~1000 mg/kg/day) gives strength to the proposed MOA, which is likely to only be operative in female Wistar rats that experience a very large lifetime deficit in body weight gain.

The registrant concluded that where parameters were measured in multiple studies, there is a high degree of reproducibility between studies and consistency between key events. The first key casual event in the proposed MOA, a dose-responsive decrease in body weight gain at dose levels of 1200 – 4000 ppm, was observed throughout the 2-year rat study, a 13-week rat study with a similar high dose level, and in multiple other short-term rat studies with sedaxane (Peffer and Parr-Dobrzanski, 2010). As shown in **Table 45**, the divergence from the control group values accelerated in the second year of the study at the 3600 ppm dose level. This degree of effect on body weight was not attained in the non-tumorigenic 1200 ppm dose level.

Downstream key events also had multiple sets of data to support them as suggested by the registrant. Retention of functional TIDA neurons in 2-year rats was suggested by higher levels of both TH mRNA and protein in the 3600 ppm group *vs* controls. Also, the associative event of decrease pituitary proliferative effects was an indirect marker of continued dopamine production by the TIDA neurons at 2 years in the 3600 ppm. As a product of continued dopamine release by the TIDA neurons, prolactin release by the pituitary remained low, which was supported by a very marked absence in mammary fibroadenomas (prolactin-driven) and decreases in other proliferative changes of the mammary gland at 3600 ppm.

Finally, as concluded by the registrant, results of the original histopathology evaluation of all tissues in the context of a 2-year cancer bioassay by CRL (Perry, 2010b) (MRID 47473386) were reconfirmed, and expanded, by a retrospective, blinded evaluation of the histology in vagina, ovaries and uterus to determine cycle stage and reproductive senescence status (MRID 49804814). The treatment-related decrease in vaginal mucification in 3600 ppm rats at 2 years

(*i.e.,* indicating fewer rats in the senescent stage of repetitive pseudopregnancy) observed in the original pathology examination (**Table 49**) was confirmed in the retrospective, blinded evaluation (**Table 50**).

Data in these studies and in the literature (Kachi *et al.*, 2006) indicate that the critical period where Wistar rats transition from normal cycling to irregular cycling to ultimately reproductive senescence is likely somewhere between 53 – 104 weeks. The confirmation that 3600 ppm rats differed in their senescent staging at 2 years compared to the control and 1200 ppm groups by a retrospective, blinded evaluation is evidence of reproducible data that suggests a change in the progression into reproductive senescence did occur in the 3600 ppm sedaxane-treated rats, as stated by the registrant.

E1. Biological Plausibility and Coherence

As recommended in an IPCS Framework analysis (Boobis *et al.*, 2006), this section considers whether the MOA is consistent with what is known about carcinogenesis in general (biological plausibility) and also in relation to what is known for the test substance specifically (coherence), including structure-activity relationships or similar treatments that produce tumors *via* the same mechanism.

According to the registrant, the uterine tumor MOA for sedaxane is proposed to occur *via* an initiating event involving large decreases in body weight gain (-50%) over the full 2-year span of this study in female Wistar rats, which is supported by a body of literature on caloric restriction and tumor profiles. In addition, the proposed MOA appears to be critically dependent on the strain of rat, based on known differences in the timing and preferred state of transition into reproductive senescence for different strains of rat (*e.g.,* Sprague-Dawley rats prefer persistent estrus while the predominant stage for Fischer-344 rats is repetitive pseudopregnancy).

E2. Large body weight effects, caloric restriction, carcinogenesis, and reproductive senescence

A retrospective examination of the effects of excessively high dose levels in chronic bioassays in rodents has demonstrated that large body weight deficits at high dose levels are frequently associated with lower incidences of total tumors, as well as increases in longevity [reviewed in Haseman *et al.* (1997)]. In addition, long-term studies of caloric restriction in rats and mice have demonstrated a host of biochemically or hormonally-mediated effects, many of them beneficial in terms of survival or tumor outcome, when compared to *ad libitum*-fed control animals that develop obesity and adverse age-related changes in long-term studies [reviewed in Frame *et al.* (1998)]. As a result of these realizations and consistent experimental data, international consortiums and various Regulatory Agencies have revised their guidance on dose selection in long-term bioassays, to help avoid confounding effects due to large body weight deficits (Foran, 1997; U.S. Environmental Protection Agency, 2005).

In a large investigative effort that is known as the Biosure study (Roe *et al.*, 1995), various methods to achieve caloric or dietary restriction were investigated in male and female Wistar rats

to assess their impact on longevity and tumor incidences in lifetime feeding studies. One form of caloric restriction (code = LMA/LMA) consisted of *ad libitum* feeding of a low nutrient maintenance diet from weaning, through 13 weeks of treatment and then throughout the rats' normal life span (30 months). These animals were compared to a control group that was feed a standard maintenance diet *ad libitum* (code = SBA/SMA). Food consumption in the LMA/LMA group females (Group 11) was higher than controls (Group 1) ($p<0.001$) throughout the study (115 – 126% of control), but food spilling was also higher than the control group and had to be assessed carefully to estimate actual food consumption. Based on the nutrient content in the different diets, the authors calculated that despite the higher food consumption, the LMA/LMA female rats had mean daily energy intakes that were 10-20% lower than the control rats. This form of caloric restriction produced a sustained decrease in body weights, which was ≥14% of mean values for the control group throughout the study. In addition, the caloric restriction produced a shift in the tumor profile that is illustrated in **Table 54**.

TABLE 54: Tumor Profile in Calorie-Restricted Female Wistar Rats from Roe *et al.* (1995)

	Incidence (Females) and Statistical Significance[a]	
	Control Group (SBA/SMA) = Group 1	**Restricted Calorie Group (LMA/LMA) = Group 11**
Mammary Gland (N):	(50)	(50)
Fibroadenoma[a]	17	4***
Anterior Pituitary (N):	(50)	(50)
Adenoma[a]	33	23***
Uterus (N):	(50)	(50)
Adenocarcinoma[a,b]	0	7**
combined - "glandular tumors of uterine horn or body"[c]	6%	20.5%**

*, **, *** $p<0.05$, $p<0.01$, $p<0.001$

[a] Statistics in Roe *et al.* (1995) were based on overall tumor incidence in that tissue; values shown above are the major contributor to the tumor incidences in that tissue. Statistics for pituitary tumors were only conducted by the authors across both sexes; similar incidences were seen in males and in females ($p<0.001$).

[b] Incidence of adenocarcinoma in "protocoled tissues" is shown as reported; it was not statistically analyzed. However, the statistical significance of the total glandular tumors (next row) has been assigned to this row (**) for presentation purposes.

[c] Text describes statistics conducted on "glandular tumors of uterine horn or body" (adenocarcinoma, anaplatic carcinoma, adenomatous polyp and papillary adenoma).

The overall incidence of benign or malignant tumors was reduced significantly ($p<0.001$) in the LMA/LMA females compared to the control group. As shown in **Table 54**, this incidence included a statistically significant decrease in both anterior pituitary adenomas and mammary gland fibroadenomas. However, uterine tumors (primarily adenocarcinomas) were significantly increased in the restricted calorie rats compared to the controls.

In a different study by Tucker (1979), Wistar-derived male and female rats were divided into two groups (50 males and 50 females per group), an *ad libitum* fed control group and a restricted calorie group whose diet availability was limited to achieve approximately 20% of the control

group value (in g food/rat/day). At the end of 24 months, the survival in the restricted calorie group of females (44%) was numerically higher than the control females (34%), but the difference was not statistically significant. In the females, specifically, there was a statistically significant reduction in the total tumor burden in the restricted calorie group compared to control. The patterns of tumors observed in the female rats were:

- Significantly lower incidences of mammary gland tumors in the restricted calorie group (3) compared to the control group (17) ($p<0.001$)
- Significantly lower pituitary tumors in the restricted calorie group (19) compared to the control group (33) ($p<0.005$)
- Higher numeric incidences of uterine tumors in the restricted calorie group (3) compared to the control group (1). All of these tumors were carcinomas, likely endometrial adenocarcinomas based on the typical background tumors described for Wistar rats in later publications.

In a review paper published more recently, Harleman *et al.* (2012) examined the incidence and coincidence of uterine tumors and mammary tumors in two different strains of rats (Wistar and Sprague-Dawley). The RITA database represents company-sponsored database of historic tumor data from the control groups of guideline toxicology studies. Syngenta Crop Protection, LLC is one of the companies that participates in the RITA database. In their review, Harleman *et al.* (2012) reviewed the incidence and coincidence of mammary, pituitary and uterine tumors in a total of 5419 Wistar rats and 2158 Sprague-Dawley rats. The authors did not differentiate between mammary gland fibroadenomas (prolactin dependent) and adenomas /carcinomas (estrogen-dependent). The results of their analysis showed the following:

- Wistar rats had a markedly lower incidence of mammary tumors (24%) than Sprague-Dawley rats (58%)
- Conversely, Wistar rats had a higher incidence of uterine tumors (5%) than Sprague-Dawley rats (0.9%)
- For Wistar rats, there was strong evidence of an inverse relationship between mammary tumors and uterine tumors (chi-square = 14.364; $p<0.001$). In contrast, although there was some evidence of an inverse relationship, a statistically significant correlation was not established for Sprague-Dawley rats, most likely driven by the higher incidences of mammary gland adenocarcinomas often observed in Sprague-Dawley strain (see below for details).
- Wistar rats that did not have a mammary tumor were significantly more likely to have a uterine tumor, and vice versa. The factor driving this response in Wistar rats was concluded to be prolactin, which increases with age due to reduced dopamine production in the hypothalamus (Greaves, 2007; Hargreaves and Harleman, 2011; Keenan *et al.*, 1996).

Harleman *et al.* (2012) also reviewed the hormonal regulation of the reproductive system in rats, in particular as it relates to changes during reproductive senescence and the onset of age-related tumors. There are fundamental differences in rats *vs.* humans in the onset of reproductive senescence and the hormonal mechanisms that accompany this transition.

Moreover, as mentioned above, there is a difference in mammary tumor types in Sprague-Dawley rats as compared to Wistar rats due to the differences of these two strains in their progression into reproductive senescence. Prior studies have more fully characterized the change from regular cycling into senescent stages for Sprague-Dawley rats and Fischer 344 rats (Eldridge *et al.*, 1999; Lang, 1990; Wetzel *et al.*, 1994). In Sprague-Dawley rats, the females are prone to change to an irregularly cycling state after 6-9 months, and their progression moves from prolonged diestrus in the initial few months to a predominant incidence of persistent estrus thereafter. As a result, Sprague-Dawley rats experience a high exposure to estradiol unopposed by progesterone during these persistent estrus weeks which leads to a high incidence of estrogen-dependent mammary adenomas and carcinomas. Fischer 344 rats do not experience a change to persistent estrus as a predominant state during senescence, and their incidence of spontaneous mammary adenomas and carcinomas is lower than Sprague-Dawley rats. In contrast, mammary fibroadenomas are prolactin-dependent (Meites, 1972; Tucker, 1997).

In addition, caloric restriction also leads to profound effects on reproductive fitness and senescence in rats. In female rats in which caloric restriction is initiated early in life (~ 45 days of age) and continued until 6 – 16 months of age, reproductive senescence was delayed in these rats compared to controls, as indicated by fertility at ages between 16 and 24 months (Osborne *et al.*, 1917). Keenan *et al.* (1995) also confirmed the markedly delayed reproductive senescence in female rats on caloric restriction diets.

Regarding structure-activity relationships to other molecules that are similar to sedaxane, the Syngenta fungicide isopyrazam at a high dose level of 3000 ppm (233 mg/kg/day) also produced a characteristic shift in the tumor profile in Wistar rats (U.S. Environmental Protection Agency, 2011b), including the following:

- A statistically significant decrease in mammary fibroadenomas (4/64) *vs.* control (14/63)
- A numerically lower incidence of anterior pituitary adenomas (24/64) *vs.* control (33/64)
- A statistically significant increase in uterine endometrial adenocarcinomas (15/64) *vs.* control (1/64).

Also consistent with the MOA for sedaxane, the high dose of isopyrazam produced a large, sustained decrease in body weight gain, at approximately 40% lower than the control group by the end of the study. Based on these very similar profiles, the registrant concluded that the MOA for uterine tumors with isopyrazam is the same as sedaxane. This set of results for a structurally similar SDHI fungicide adds further weight to the biological plausibility of the proposed MOA for sedaxane.

E3. Normal reproductive senescence in the Wistar female rat

As a second topic that is important to the biological plausibility of the proposed MOA for sedaxane, the normal biology of reproductive cycles and the transition into senescence by the Wistar rat has been described in the literature, and results in this Weight of Evidence document for sedaxane are consistent with that known biology.

As with any species, the normally aging female Wistar rats undergo a series of changes with increasing age, which involves normal age related increases in body weight as well as progressive changes to the hypothalamic – pituitary – gonadal (HPG) axis which in turn affects the hormonal milieu. As Wistar rats age, plasma prolactin levels increase which corresponds to an age-dependent higher incidence of pituitary hyperplasia and tumors (Greaves, 2007; Tucker, 1997). In particular, the tuberoinfundibular dopaminergic (TIDA) neurons show a decrease in the number of dopamine-producing neurons and a decrease in the amount of dopamine that is released into the portal capillaries, which carry dopamine to the anterior pituitary. Dopamine exerts tonic inhibitory effect on the release of prolactin from the anterior pituitary gland; therefore, the decreased levels of dopamine result in higher levels of circulating prolactin. Prolactin is luteotropic in rats, promoting production of progesterone in the corpora lutea after ovulation, and sustained higher levels of prolactin (*i.e.,* in pregnancy or in repetitive pseudopregnancy) maintains the corpora lutea for longer periods of time. During the luteal phase of the normal rat 4-day reproductive cycle, progesterone is produced in large quantities by the corpora lutea (CL), which antagonizes estrogenic stimulation of uterine growth (Gambrell *et al.*, 1983). As a normal female Wistar rat goes into reproductive senescence, the loss of the tonic inhibition of prolactin release leads to higher baseline blood levels of prolactin and a progesterone dominance (*i.e.,* estrogen/progesterone ratio is lower).

In addition, prolactin plays an important role in maintaining normal reproductive cycling in the rat (Freeman, 2006; Grattan and Le Tissier, 2015), and therefore, perturbed prolactin release (*i.e.,* loss of tonic inhibition *via* loss of dopaminergic activity) leads animals into reproductive senescence. In Wistar rats, where repetitive pseudopregnancy appears to be the predominant initial senescent state (Kachi *et al.*, 2006), the influence of prolactin on the CL and reproductive senescence favors higher levels of progesterone release by the CL, and thus a lower estrogen: progesterone ratio. These hormonal and physiological changes in aging Wistar rats produce the following histological changes:

- An increase in pituitary proliferative changes (including adenomas), due to the increased activity of pituitary lactotrophs caused by lower dopamine release from the TIDA neurons.
- An increase in mammary fibroadenomas, due to the increase in circulating prolactin.
- A protective effect on the uterine endometrium, due to diminished estradiol: progesterone ratios and resulting lower proliferative signaling.

Additional literature reviews provide good evidence for the biochemical changes that occur, and the hormonal changes that are specifically observed in Wistar rats. It is well understood that most rat pituitary hyperplasia's and adenomas are prolactin positive (Kovacs *et al.*, 1977) and are functional prolactin-producing tumors. Rats with pituitary adenomas have high levels of circulating prolactin which correlates with the size of the pituitary tumor (Greaves, 2007). Because prolactin is the major promoter of mammary gland fibroadenomas due to its trophic function, there is a direct correlation between circulating prolactin and the incidence of mammary fibroadenomas (Tucker, 1997; Welsch *et al.*, 1970). Therefore, substances that stimulate the anterior pituitary and increase the levels of prolactin in blood are associated with an increased incidence of pituitary and mammary tumors (Gopinath *et al.*, 1987; Greaves, 2007).

On the other hand, chemicals that reduce prolactin secretion by acting as dopamine agonist, such as bromocriptine, cause a reduction in the incidence of pituitary hyperplasia and adenomas as well as a significant reduction in the incidence of mammary fibroadenomas (Griffith, 1977; O'Connor *et al.*, 2000). Caloric restriction in Wistar rats has been shown to reduce pituitary hyperplasia with a reduced incidence of pituitary adenomas as well as a reduction in mammary tumors, which were linked to the decreased levels of circulating prolactin (Roe *et al.*, 1995).

As discussed in the previous section, a decrease in mammary fibroadenomas and anterior pituitary adenomas plus an increase in uterine adenocarcinomas have been reproducibly demonstrated in caloric restriction studies in Wistar rats. In contrast, this spectrum of changes in the presence of large body weight deficits does not appear to happen in other strains (*i.e.,* Sprague-Dawley rats do not demonstrate an increase in uterine tumors compared to controls) (Keenan *et al.*, 1996). The typical phases of reproductive senescence to which a particular strain of rats is preferentially exposed (*e.g.,* persistent estrus for Sprague-Dawley rats *vs*. repetitive pseudopregnancy for Wistar rats) correlate with the observed strain differences in uterine adenocarcinoma incidence and other tumor types in control *vs.* calorie-restricted rats. These changes in tumors profiles have been demonstrated to be related to prolonged retention of normal dopamine levels in specific regions of the hypothalamus in calorie-restricted rats, which prevents the age-related increase in circulating prolactin that is seen in *ad libitum*-fed control Wistar rats.

E4. Conclusions – Biological plausibility and coherence

To summarize, the registrant concluded, in Wistar rats, multiple studies of caloric restriction or chemical – induced large body weight deficits in lifetime studies have been shown to produce a characteristic shift in the incidences of certain specific tumor types, including an increase in uterine adenocarcinomas (Harleman *et al.*, 2012; Roe *et al.*, 1995; Tucker, 1979; U.S. Environmental Protection Agency, 2011b). Therefore, the registrant concluded that a strong scientific precedence for the postulated MOA with sedaxane has been established, and the proposed key events in this MOA are consistent with the known biology in Wistar rats.

F. Alternative mode of action hypotheses

In addition to the MOA described (**Figure 9**), alternative modes of action have been proposed by the registrant for the induction of uterine tumors. The plausible alternative MOAs for rat uterine tumors with sedaxane treatment of Wistar rats have been considered, and data which supports or refutes these alternative MOAs are described below.

Sedaxane is not genotoxic

This MOA can be excluded as sedaxane has been demonstrated to be negative for genotoxicity in a comprehensive package of *in vitro* and *in vivo* assays for genotoxicity (**Table 28**).

In its prior review of the *in vitro* and *in vivo* genotoxicity studies, US EPA concluded that sedaxane was negative in the mutagenicity studies (U.S. Environmental Protection Agency, 2013). An additional study (MRID 50102201) is being submitted along with this current cancer weight of evidence document, which confirms that sedaxane is not genotoxic.

Sedaxane is not estrogenic

Compounds that can mimic the normal ligands that bind to and activate the estrogen receptors (primarily ERα and ERβ) are known to cause an increase in uterine endometrial tumors (Sherman, 2000; Yoshida *et al.*, 2012). This estrogenic effect can occur *via* binding of the parent xenobiotic molecule to the estrogen receptor, or after conversion to metabolite(s) that can bind and activate the receptors. To investigate the potential for sedaxane to operate *via* this MOA, an OECD Guideline 440 Uterotrophic Assay (US EPA Guideline OPPTS 890.1600) was conducted in ovariectomized (OVX) Wistar rats (strain designation Crl:WI(Han)) (MRID 49804803). In summary, sedaxane was not estrogenic in an *in vivo* uterotrophic assay in female Wistar rats. In combination with the wider database of mammalian toxicology studies (Peffer and Parr-Dobrzanski, 2010), in which sedaxane did not show any histopathology or organ weight changes that would be expected from an estrogenic substance (or its metabolites), it can be concluded that sedaxane does not show the potential to produce uterine proliferative changes *via* an estrogenic MOA.

Sedaxane is not a dopamine agonist

Compounds that act as dopamine agonists, such as bromocriptine, can decrease prolactin levels in rats. With bromocriptine, a 100-week study in male and female rats (strain not specified) was conducted as part of the safety testing requirements for this human drug (Richardson *et al.*, 1984). In female rats, bromocriptine caused a statistically significant increase in uterine adenocarcinomas at 9.9 and 44.5 mg/kg/day and a decrease in mammary tumors (specific types not mentioned) in all treated groups. Thus, dopamine agonists have been shown to increase the incidence of uterine tumors in rats by suppressing prolactin and altering the pattern of estrous cycling or the transition into reproductive senescence (Griffith, 1977; Klaunig *et al.*, 2015; Richardson *et al.*, 1984).

To investigate the potential for sedaxane to act as a dopamine agonist, it was tested in a competitive binding assay with a human dopamine receptor (type D2S). In summary, it was suggested that sedaxane was inactive for binding to the dopamine receptor *in vitro* (MRID 49804817). In addition, the wider toxicology database for sedaxane [summarized in Peffer & Parr-Dobrzanski (2010)] did not display the same types of short-term toxicity as seen in preclinical studies with bromocriptine that would suggest a short-term, pharmacological effect as a dopamine agonist [summarized in Richardson *et al.* (1984)]. Therefore, an alternative MOA for rat uterine tumors *via* activity as a dopamine agonist has been excluded for sedaxane.

Exclusion of other potential modes of action

More recently, several authors have indicated that additional MOAs may be operative for rat uterine adenocarcinomas, based on initial key events such as induction of specific CYP enzymes involved in estradiol metabolism or inhibition of enzymes related to estradiol conjugation and excretion (Wikoff *et al.*, 2016; Yoshida *et al.*, 2015). These MOAs involve short-term effects on metabolism or neuroendocrine systems that result in a higher net estrogenic stimulation of the uterus, and would be expected to produce changes in estrogen-sensitive tissues and/or related estrogen pathway endpoints after short-term as well as long-term administration. Since sedaxane did not produce any changes in the uterus, ovaries, vagina or other similar tissues in rats, mice,

dogs and rabbits during *in vivo* studies of 3 days through 1 year in duration (Kappeler, 2014 (MRID 49804803); Peffer and Parr-Dobrzanski, 2010), these short-term MOAs are not operative for sedaxane.

G. Uncertainties, inconsistencies and data gaps

Based on the guidance regarding the IPCS Framework (Boobis *et al.*, 2006), uncertainties should include those related to both the biology of tumor development and those for the database on the compound of interest. Inconsistencies should be flagged and data gaps identified. For the identified data gaps, there should be some indication of whether they are critical as support for the postulated MOA.

The registrant concluded that the available data support the proposed hypothesized MOA for the higher incidence of rat uterine tumors with sedaxane (**Figure 9**), while excluding the alternative MOAs described above. There is a strong database in the literature for caloric restriction where similar deficits in body weight of the magnitude seen with sedaxane have produced the same shift in tumor profile in Wistar rats. The data points for sedaxane, as summarized in the Dose-Concordance Table (**Table 52**) provide either direct evidence for the key event, or associative events that can serve as markers for each key event.

One potential data gap that exists is a lack of data specifically for sedaxane-treated female Wistar rats showing the proposed key event of decreased adipose tissue. In the 104-week chronic/carcinogenicity study in rats, specific measurements that would reflect a decrease in the percentage of adipose tissue (*e.g.,* abdominal fat pads, omental fat) were not a routine part of the study design. Therefore, a direct measure of decreased adipose tissue in the 3600 ppm female rats was not obtained. However, based on known responses in rat studies to caloric restriction, and the increasing percentage of body weight in obese rats that is represented by fat at the end of a 2-year *ad libitum* feeding study, it can be presumed, by the registrant, that 3600 ppm sedaxane-treated female rats that had significantly lower body weights than controls (-33% at 104 weeks) would also have lower adipose tissue as a percentage of total body weight compared to controls.

For example, Wolden-Hanson *et al.* (1999) studied the progression of adipose tissue deposition in male Brown Norway rats from age 3 to 29 months. In this study, the total body fat rose with age faster than the body weight of the *ad libitum* fed rats, such that the percentage of body weight as fat increased from 8.8% at 3 months of age to 19.9% at 29 months of age. While the weight of the visceral fat pads increased also with age, it progressively represented a lower % of total fat. The peripheral fat was proportionately a higher % of total fat with increasing age. Leptin levels in the blood progressively increased in proportion to the increase in percentage of body fat as the rats aged. Leptin and adiponectin levels in blood are known to be directly proportional to fat deposition levels, and they influence appetite and other energy-balance signals *via* receptors in the hypothalamus and other regions of the brain (Arner, 2003; Tena-Sempere, 2015; Woodside *et al.*, 1998). In addition to leptin and adiponectin, other signaling factors or metabolism products (*e.g.,* ketone bodies, sirtuins, cAMP responsive element binding (CREB))

have also been shown to change in diet restriction studies, and multiple studies have linked these changes during diet restriction to a neuroprotective effect (Lin *et al.*, 2015; Pani, 2015).

In a study of the effect of caloric restriction on tumor profiles in Wistar rats (Roe *et al.*, 1995), decreases in body weights compared to *ad libitum* fed controls (code = SBA/SMA) were observed in female rats on a high-fiber restricted calorie regimen for 30 months (code = LMA/LMA). At the end of the study, the LMA/LMA caloric-restricted female rats had body weights that were -14% different from the *ad libitum* fed controls. This regimen produced an increase in uterine adenocarcinomas, and decreases in mammary fibroadenomas and pituitary adenomas (as discussed in a previous section on Biological Plausibility). The authors did not quantify the weight or percentage of body fat in the different feeding regimens, but they noted in their discussion that restricted animals were not insulated by the thick layer of fat in the body wall (*i.e.,* peripheral fat), as opposed to *ad libitum* fed rats, which may have caused them to use more energy to maintain body temperature.

In summary, published experimental data in rats has established that increasing fat deposition occurs with age in *ad libitum* fed rats, and that caloric restriction studies that produced body weight deficits (-14%) smaller than those seen in the 3600 ppm sedaxane females (-33%) resulted in less deposition of fat than in the *ad libitum* fed control rats. Therefore, the registrant stated that it is reasonable to conclude that the 3600 ppm sedaxane-treated female rats had lower percentages of body fat than the control rats. Therefore, the lack of actual data related to adipose tissue content in sedaxane-treated rats does not detract from the overall weight of evidence for the proposed MOA.

Another potential data gap for the sedaxane MOA is the key event regarding the increased age at reproductive senescence. The proposed key event is that the high dose group experiences more estrus cycles in its lifetime, which leads to a greater cumulative exposure of estrogens to the uterus that ultimately leads to uterine endometrial proliferation (**Table 53**). To definitively demonstrate this key events, it would be necessary to determine cycle stage by continuous vaginal lavages throughout the lifestage of the Wistar female rat, specifically between 53 and 104 weeks of a 2-year chronic study. Continuous vaginal lavages between 53 and 104 weeks would allow for determination of the timing as well as progression of changes in estrous cycling that eventually leads to persistent anestrus. For example, some Wistar rats may experience irregular cycles, followed by repetitive pseudopregnancy, followed by persistent anestrus, while others may proceed from irregular cycles directly to persistent anestrus (vom Saal and Finch, 1988). In addition, histopathology examination of the reproductive organs (*i.e.,* vagina, ovaries, uterus) of a sufficiently large number of rats across a series of interim sacrifice time intervals from 53 – 104 weeks may be necessary to further confirm the cycle stage as indicated by the vaginal lavages. However, for dose concordance of the key events (**Table 52**), the registrant concluded that ample data is available with sedaxane for an Associative Event that provides a suitable marker for the reproductive senescence related key event. The registrant concluded that for the terminal sacrifice time point of 104 weeks, the original pathology results (**Table 49**) and a retrospective, blinded histology evaluation (**Table 50**) detected a lower incidence of senescent

mucification (repetitive pseudopregnancy) at the high dose of 3600 ppm. In contrast, there were no effects on any of these markers at 200 or 1200 ppm. In summary, the registrant concluded that these datasets provide ample evidence for an Associative Event that helps to confirm that the key event(s) related to the age of reproductive senescence were also perturbed by treatment with 3600 ppm sedaxane at some point between 53 and 104 weeks. Therefore, the registrant stated that this potential data gap is not critical to the weight of evidence for this MOA, and there is supporting literature evidence that caloric restriction has profound effects on reproductive senescence in rats (Keenan *et al.*, 1995; Osborne *et al.*, 1917). In addition, a clear dose response has been suggested for the measurable events, *i.e.,* there were no effects on these Associative Events at 1200 ppm or below.

The registrant concluded that the few data gaps or inconsistencies noted above do not diminish the strength of the evidence for the proposed MOA. No other uncertainties, inconsistencies or data gaps have been identified.

XIII. ASSESSMENT OF THE POSTULATED MODE OF ACTION FOR UTERINE TUMORS

The registrant concluded that the concordance analyses have established that the proposed key events resulting in a higher incidence of uterine tumors in female Wistar rats exhibit good dose- and temporal-concordance with the tumor endpoint. This MOA for the induction of uterine tumors with sedaxane has ample precedence in the literature in terms of the shift in tumor profiles that occurs in Wistar rats when body weights are sufficiently impaired due to caloric restriction (Harleman *et al.*, 2012; Roe *et al.*, 1995; Tucker, 1979; U.S. Environmental Protection Agency, 2011b), and the associated precursor key events in rats, and the parameters essential for describing the MOA have been presented for sedaxane. In addition, alternative MOAs for generating uterine tumors in rats have been examined and excluded for sedaxane based on further experimentation. Therefore, there is a high level of confidence, as stated by the registrant, that the hypothesized MOA (**Figure 9**) is responsible for the higher incidence of uterine tumors in female rats following dietary exposure to 3600 ppm (261 mg/kg/day) sedaxane.

XIV. ARE THE KEY EVENTS IN THE ANIMAL MODE OF ACTION FOR UTERINE TUMORS PLAUSIBLE IN HUMANS?

Following establishment of a plausible MOA for the induction of uterine tumors in rats, the next step is to assess the relevance to humans by assessing the qualitative and quantitative differences between the rat and human for each of the key events.

With regard to the hormonal control of the HPG axis, and the changes that occur in the transition from normal reproductive age into reproductive senescence, there are fundamental differences between rats and humans that are critical to evaluating the potential relevance of the MOA that is established for sedaxane-treated rat uterine tumors.

The human reproductive cycle (menstrual cycles) has very different control mechanisms compared to rats (4-5 day estrous cycles) (**Table 55**). First, the surge of prolactin during proestrus in rats is not observed in human menstrual cycles. Second, the normal luteal phase of a rat estrous cycle is short (~1 day), and the new corpora lutea (CL) will regress, but if a high prolactin level is maintained (*e.g.,* a twice per day surge of prolactin occurs upon the stimulation of the cervix in mating), this higher prolactin will rescue the new CL and stimulate it to produce sustained higher levels of progesterone. In contrast, human CLs are initially maintained by LH (during a menstrual cycle), and later by chorionic gonadotropin from the placenta when pregnancy occurs. In rats, but not humans, luteolysis is also under partial control by prolactin, which is thought to be mediated by recruitment of macrophages in the ovary to degrade prior CLs (Freeman, 2006; Grattan and Le Tissier, 2015; Harleman *et al.*, 2012).

TABLE 55: Regulation of Reproductive Cycles in Rats and Humans

	Rat	**Human**
Prolactin surge	Occurs in proestrus	No significant changes during the menstrual cycle.
Luteal phase length	Short (~1 day)	Long (14-16 days)
Role of Prolactin in Luteal phase	Luteotrophic which rescues new CLs and ↑ Progesterone synthesis	None – mediated by LH (initially) + chorionic gonadotropin (in pregnancy).
	Luteolytic which recruits macrophages to degrade prior CLs – prolactin surge in proestrus	None

References: Freeman (2006); Grattan and Le Tissier (2015); Harleman *et al*. (2012).

Similar to the fundamental differences between rats and humans in the control of normal cycling or the control of pregnancy, reproductive senescence processes are also fundamentally different (**Table 56**). In Wistar rats, onset of senescence is driven by a progressive decrease in the activity of dopaminergic neurons in the hypothalamus, particularly the TIDA neurons. In contrast, menopause and reproductive senescence in humans is driven by an eventual depletion of a limited number of primordial follicles in the ovaries with age. In Wistar rats, as a consequence of the loss of the dopamine-mediated tonic inhibition, prolactin levels in the blood are elevated which results in a luteotrophic effect on the CLs, and this in turn results in elevated progesterone and lower estrogen in the blood. This regulation does not occur in humans. Human female reproductive senescence occurs at the level of the ovaries, since the onset of menopause is driven by depletion of the limited number of available follicles within the ovaries (**Table 56**). In addition, it is well known that menopause in human females is associated with a marked decrease in circulating estrogens and progesterone.

TABLE 56: Reproductive Senescence in Rats and Humans

Parameter	Wistar Rat	Women
Principal cause of senescence	Hypothalamic failure to control prolactin surges – loss of dopaminergic function	Depletion of ovarian follicle content – [hypothalamic function is maintained]
Predominant cycle pattern	Repetitive Pseudopregnancy	Menopause
Estrogen/progesterone ratio during this senescent stage	Reduced	Reduced
Prolactin secretion in reproductive senescence	Elevated	Reduced
Dopaminergic control	Yes	No
Prolactin dependence	Medium	None

References: Freeman (2006); Grattan and Le Tissier (2015); Harleman *et al.*.(2012); vom Saal and Finch (1988); Neal-Perry and Santoro (2006).

The main stages of senescence in rats can be species-dependent; in Wistar rats, the aging animals proceed mainly into irregular cycles, followed by repetitive pseudopregnancy and eventually persistent anestrus. In repetitive pseudopregnancy, a higher baseline level of progesterone production by the CLs of the ovary achieves a low estradiol: progesterone ratio.

The registrant concluded that compared to control rats, the large body weight gain deficits that can be produced in rats by calorie restriction or by treatment with 3600 ppm sedaxane have been shown to preserve the TIDA neurons in aging Wistar rats, which leads to continued low prolactin levels, longer continued estrous cycling and periodic exposure to estradiol unopposed by progesterone (*i.e.*, a higher estradiol: progesterone ratio). The increased time of exposure to a higher estradiol: progesterone ratios leads to a higher incidence of uterine tumors in Wistar rats. This MOA is not relevant to humans, since the pathways leading to reproductive senescence and the control of the HPG axis in humans are not mediated by dopaminergic signaling.

Moreover, caloric restriction influences the progression into reproductive senescence in rats. However, in non-human primates, these changes are not observed with caloric restriction. In female rhesus monkeys, long-term caloric restriction had no effect on menstrual cycling or age-related decline in menstrual cycling (Lane *et al.*, 2001).

In summary, a wealth of information in the literature has suggested that the key events that lead to uterine tumors in Wistar rats after large, sustained decreases in body weight (*i.e.,* as seen with 3600 ppm sedaxane) would not be operative in humans, because of fundamental species differences in the control of reproductive cycles and the transition into reproductive senescence. Therefore, as stated by the registrant, based on qualitative differences, the MOA established in rats with sedaxane is not relevant to humans.

XV. REGISTRANT UTERINE TUMOR CONCLUSIONS

The registrant concluded that available data for sedaxane support the proposed MOA (**Figure 9**) with an explanation for this conclusion provided hereinafter. The higher incidence of uterine tumors in female rats is attributable to a large deficit in body weight, which results in changes/delay in reproductive senescence by preserving the dopaminergic neurons of the hypothalamus. The continued high dopamine activity has a tonic inhibitory effect on prolactin release by the pituitary. Specifically, for Wistar rats, this change (mediated *via* a state similar to caloric restriction) compared to normal aging control rats leads to a lower incidence of tumors in the pituitary and mammary glands, and a higher incidence of uterine adenocarcinomas. This same pattern of changes in Wistar rats has been demonstrated to occur in rats maintained for their lifetimes on a restricted calorie diet. The suppression of the age-related increases in prolactin levels by sustained dopamine activity results in changes/delay in reproductive senescence and consequently greater cumulative exposure of the uterus to a higher estrogen: progesterone ratio (*i.e.,* reduced progesterone dominance of estrogen) in aged female rats, which would lead to a pro-proliferative estrogenic stimulation of the uterine endometrial cells. Over time, the estrogenic proliferative drive leads to promotion of spontaneously initiated uterine adenocarcinomas. At the same time, the decreased prolactin signaling leads to decreased proliferation of the anterior pituitary and mammary glands, which in turn leads to lower incidences of pituitary adenomas and prolactin-driven mammary gland fibroadenomas. The control of the female reproductive cycles and the drivers for reproductive senescence in humans are fundamentally different than that in rats, and therefore, this MOA for uterine tumors in rats is not relevant to human risk assessment due to qualitative differences between the species.

Clear thresholds exist for the key events in this MOA. Based on all of the factors described in this document, a linear low-dose model (Q1*) is not appropriate for human cancer risk assessments. In addition, the control of the female reproductive cycles and the drivers for reproductive senescence in humans are fundamentally different than those in rats; therefore, this MOA for uterine tumors in Wistar rats is not relevant to human risk assessment due to qualitative differences between the species. Thus, through the weight of evidence evaluations, the data show that sedaxane is “not likely to be carcinogenic to humans”.

XVI. COMMITTEE’S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE FOR THE MOA FOR UTERINE TUMORS IN FEMALE RATS

On February 22, 2017, CARC reconvened to evaluate the submissions of new studies containing MOA data on sedaxane. From these deliberations, the CARC drew the following conclusions for the sedaxane-induced rat uterine tumor MOA:

- **The proposed initial key event (a decrease in body weight) does not define a molecular interaction or initiating event. Moreover, it was not clear why uterine tumors would not be observed more commonly for other chemicals that decrease body weight.**

- **The second key event (a decrease in adipose tissue) is not directly supported by the evidence provided.**

- **The evidence supporting the third key event (suppression of an age-related decrease in dopaminergic signaling) had the following weaknesses: (1) There was not significantly lower tyrosine hydroxylase (TH) protein in the hypothalamus (as marker of dopaminergic neurons) at 2 years compared to 90 days of age in the control experiment (MRID 49804815). (2) While sedaxane increased TH expression in the TIDA region at 2 years, this does not necessarily indicate a suppression of an age-related decrease in dopaminergic signaling. To properly do this analysis one would need to analyze the data as a 2x2 factorial with time and treatment and look for an interaction.**

- **While the decrease in mammary fibroadenomas is suggestive, the fourth key event (suppression of age-related increase in prolactin) was not directly supported by serum prolactin or other data (MRID 50101901). There is also no direct evidence supporting an association between dopaminergic activity and prolactin levels.**

- **There was a modestly lower incidence of vaginal mucification in the high-dose group (3600 ppm) but this effect was not statistically significant from controls on the histopathology re-evaluation, and there were no clear differences in cyclicity or overall senescence at 1 or 2 years of age.**

- **An increase in the number of estrous cycles would not necessarily be associated with an increase in unopposed estrogen exposure (*i.e.*, without progesterone). Moreover, no evidence for an increase in estrogen:progesterone balance was demonstrated.**

- **There was no evidence of squamous metaplasia, endometrial hyperplasia, or other evidence of increased estrogenic exposure lesions at one or two years. Thus, the primary growth stimulus for uterine epithelial cells was not identified.**

Conclusion: Based on the deficiencies identified above, there is not sufficient evidence to support the proposed MOA for rat uterine tumors induced by sedaxane.

XVII. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), CARC concludes that sedaxane should be reclassified as "Suggestive Evidence of Carcinogenic Potential" based on uterine tumors in female rats (one sex/one species). There is insufficient evidence to support the proposed uterine tumor MOA in female rats.

The rationale for this decision is based on the following considerations:

- The liver and thyroid tumor response induced by sedaxane occurred in only male rats and/or mice; no liver or thyroid tumors were seen in female rats or mice.

- The liver tumor response in male rats occurred late in the course of treatment and was driven by adenomas; no carcinomas were observed. It was considered to be weak evidence of a treatment-related effect.

- Male mice liver tumor response was driven by adenomas and combined adenomas and/or carcinomas. However, all non-neoplastic histopathology findings were considered background findings associated with the age and strain of mice.

- The thyroid tumor response in male rats was driven mainly by adenomas; however, there was also an increase in combined adenomas and/or carcinomas. It was concluded that thyroid tumor incidence provided weak evidence of a treatment-related effect.

- There is no concern for mutagenicity.

- Data are sufficient to support the proposed MOA for liver tumors in male rats and mice and thyroid tumors in male rats. However, data are not sufficient to support the proposed MOA for female rat uterine tumors.

XVIII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The CARC determined that quantification of cancer risk using a non-linear approach (i.e., RfD) would adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to sedaxane.

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